The Occurrence and Distribution of Salmonid Viruses in Oregon

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J. L. Fryer

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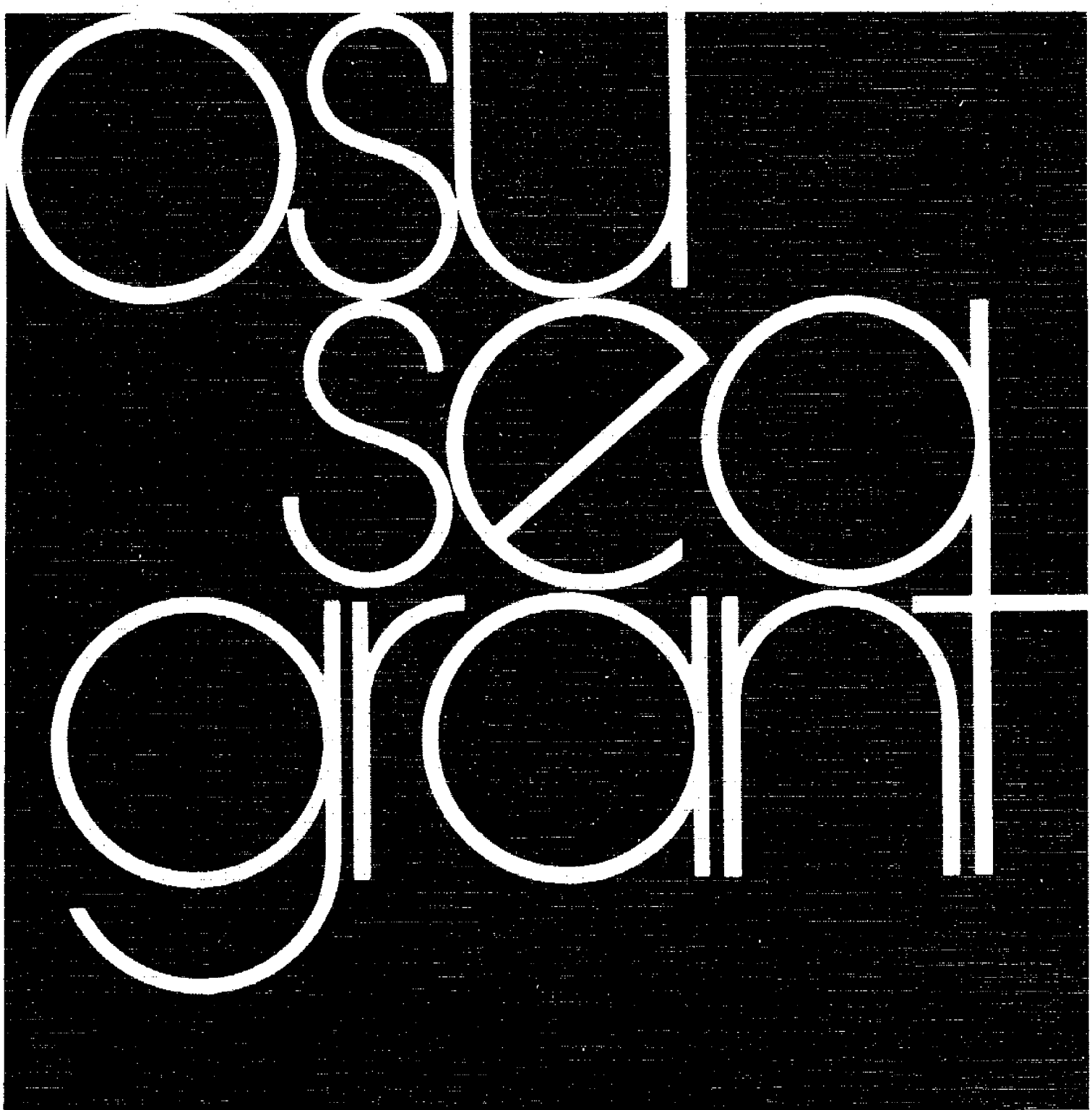

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Dedicated To

Max V. Frame

1906-1966

and

Orval E. Greer

Fish Culturists

Oregon Department of Fish and Wildlife

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foreward

A fish virus was identified for the first time in populations of Oregon's salmonids during 1958. Slightly over 20 years have passed since that original isolation. During this time, we have performed numerous examinations for the presence of viral disease, particularly from 1971 to 1977 when intensive efforts were made to determine the distribution of these agents among salmonids. As a result, Oregon's salmonids are among the most intensively studied in the world for the presence of viruses.

The history of viral examinations in Oregon's salmonids, and the incidence of viral diseases in Oregon hatcheries, may be particularly useful and interesting to fisheries administrators, fish culturists, biologists and other scientists who want information about viral isolations in the state's salmonids. This manuscript has been prepared primarily for them.

The authors wish to express their appreciation to William Q. Wick, director of the Sea Grant College Program at Oregon State University, and his staff for their willingness to publish this report, and their help.

1980

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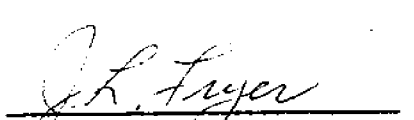

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abstract

Salmonid populations in Oregon hatcheries, rivers and lakes have been examined extensively for the presence of viruses pathogenic for these species. Cell culture methods were employed that permitted the isolation of such agents from fish tissues and their identification. Two viruses have been isolated during the course of this investigation. The first of these recovered was found to cause an epizootic among juvenile sockeye salmon *(Oncorhynchus nerka)* at the Oakridge Salmon Hatchery in 1958. The disease that the virus causes is now known as infectious hematopoietic necrosis (IHN). During the period from 1958 through 1978 the virus has been recovered 24 times[1] from fish at seven collection sites. These included four hatcheries, three rivers and a small lake. It has been responsible for significant mortality in at least eight instances.

The second virus found in Oregon's salmonids causes the disease known as infectious pancreatic necrosis (IPN). It was isolated in 1971 from adult cutthroat trout *(Salmo clarki)* resident in a small creek, and from adult coho salmon *(Oncorhynchus kisutch)* being spawned at Bonneville Hatchery on the Columbia River. In both cases the fish were asymptomatic carriers. Between 1971 and 1978, infectious pancreatic necrosis virus (IPNV) was isolated 27 times[1] from fish collected at 13 different sites. These sites included seven hatcheries, five lakes, one river and a small creek. It was the cause of at least three epizootics in which the highest mortality occurred among populations of Eastern brook trout (*Salvelinus fontinalis*).

Evidence for the presence of a third fish virus was found in the erythrocytes of spawning adult chum salmon *(Oncorhynchus keta)* and Pacific herring *(Clupea harengus pallasi)* taken in waters along the Oregon coast. Cytoplasmic particles with hexagonal profile, strongly resembling polyhedral virions, were found in the erythrocytes by electron microscopy. This virus

has not been isolated in cell cultures. The associated disease is now referred to as viral erythrocytic necrosis (VEN).

Herpesvirus salmonis, first isolated from rainbow trout *(Salmo gairdneri)* in the Pacific Northwest, has never been found among any fish population examined in Oregon.

Recommendations for the control of IHN and IPN viruses are included in this report.

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[1] Repeated recoveries of the same virus from a single collection site were arbitrarily not considered as separate isolations unless they were made at intervals of at least 14 days.

introduction

We have acquired knowledge of viral diseases in Oregon's salmonids only within recent years. The first recognized viral infection occurred in 1958 when an epizootic with a high mortality rate appeared among young sockeye salmon *(Oncorhynchus nerka)* at the Oakridge Salmon Hatchery. A virus was isolated from diseased fish by one of us (J. L. F.) and shown to be the causative agent. It is now known as the virus of infectious hematopoietic necrosis, or IHN.

In 1971 a second salmonid virus was detected in Oregon's fish for the first time. This was the agent of infectious pancreatic necrosis, or IPN. It was isolated by one of the authors (J. S. M.) from asymptomatic cutthroat trout *(Salmo clarki)* in a stream that drained a small private lake, and also from apparently healthy adult coho salmon *(Oncorhynchus kisutch)* being spawned at the Bonneville Salmon Hatchery. Although the virus was not causing obvious mortality in either case, it later produced a severe epizootic in an Oregon hatchery.

In 1976 and 1977 a third virus was detected by one of us (M. P-F.) by microscopically examining the erythrocytes of two fish species taken in waters along the Oregon coast. Large particles of hexagonal profile, having the typical appearance of polyhedral virions, were found in the cells of chum salmon *(Oncorhynchus keta)* and Pacific herring *(Clupea harengus pallasi)*. No virus has been isolated and little is known of its properties. It is referred to as the virus of piscine erythrocytic necrosis, or PEN, and most recently as viral erythrocytic necrosis, or VEN.

As soon as the presence of virulent viruses in certain salmonid populations was recognized, it became important to obtain information about the prevalence and distribution of these agents in Oregon waters. This information was needed to identify virus-free egg sources from wild fish where required; to avoid the transfer of fish carrying viruses to hatcheries or waters free from these agents; and to assess the possible

effect of a particular virus on the maintenance and welfare of Oregon's salmonid fisheries. Accordingly, salmonid populations throughout the state have been extensively studied from 1958 through 1978 for the presence of viruses. Hatcheries, rivers and lakes have all been included. It is the purpose of the present report to provide complete information concerning this survey, including the methods used and the results, and to make recommendations for hatchery practices and control measures based on the acquired information.

the viruses

INFECTIOUS HEMATOPOIETIC NECROSIS

Historical Review

The first occurrence of disease caused by what is now presumed to be the IHN virus was observed among sockeye salmon *(Oncorhynchus nerka)* in hatcheries at Winthrop and Leavenworth, Washington, and was described by Rucker et al. (1953). Watson et al. (1954) reported further experimental evidence for the viral etiology of this disease.

In 1958 an outbreak of disease with a high mortality rate appeared among young sockeye salmon at the Oakridge Salmon Hatchery in Oregon. J. L. Fryer isolated an infectious agent from the diseased fish that could transmit infection to juvenile sockeye salmon when they were injected with bacteria free filtrates of infected tissues. This agent possesses characteristics of animal viruses and is now thought to be a strain of the same virus reported by Rucker et al. (1953). The properties of this virus will be described in a subsequent section of this report.

In 1967 Amend et al. (1969) isolated a previously unreported virus in rainbow trout *(Salmo gairdneri)*. The moribund fish in this outbreak were from a private trout farm in western Canada. Shortly thereafter, they isolated a similar agent from groups of moribund sockeye salmon from a hatchery near the trout farm. Studies of these isolates indicated that they were very similar to the Oregon sockeye salmon virus, and their pronounced cytotropism for the hematopoietic tissues of the host suggested the name "infectious hematopoietic necrosis" for the disease.

Another agent, now regarded as a strain of the same virus, causes the Sacramento River chinook disease in chinook salmon *(Oncorhynchus tshawytscha)* of California. This strain, as well as those recovered from other salmonids, are now referred to as IHN virus. A number of recent isolations from Oregon salmonid populations have been made.

The work reported here emphasizes viruses isolated in cell culture and identified by serum neutralization. It is important, however, to note that J. W. Wood (personal communication, Washington Department of Fisheries, College of Fisheries, University of Washington, Seattle) had demonstrated the presence of a filterable agent in sockeye salmon prior to these observations. Wood prepared homogenates of infected fish, passed this through a porcelin type filter, and injected the filtrate back into healthy animals producing death with signs of typical IHN. This work was accomplished in about 1957.

Characteristics of the Virus

When investigating the epizootic in sockeye salmon at the Oakridge Salmon Hatchery in 1958 it was found that bacteria free filtrates of tissues from infected fish were capable of transmitting infection to juvenile sockeye by injection. The agent was found to replicate abundantly in cultures of sockeye salmon cells, producing characteristic cytopathic effects (Wingfield et al. 1969). The maximum rate of replication occurred in the 13° to 18°C temperature range, and no replication occurred at 23°C, although host cells grew well at this higher temperature. Infectivity was destroyed by ether, indicating the presence of essential lipids. Electron microscopic studies were incomplete at that time. The virus was found to replicate in the cytoplasm of the cells in culture (McAllister et al. 1974, McCain et al. 1974) and its genome was shown to be single stranded ribonucleic acid (RNA).

Amend et al. (1969) diagnosed the disease in rainbow trout from a private trout farm in British Columbia and in sockeye salmon from a nearby hatchery. Electron microscopy of the isolated agents revealed bullet shaped virions with a mean length of 188 nm and a diameter of 70 nm (Amend and Chambers 1970). These observations have been confirmed and have resulted in classifying the virus as a member of the rhabdovirus group, which contains all known bullet shaped viruses. Figure 1b shows an electron micrograph of the virus.

Pathology

In juvenile sockeye salmon, where the disease was first observed, one of the most characteristic signs of infection is the presence of long, opaque fecal casts trailing from the vent. Ascites, exophthalmos and hemorrhagic areas in the musculature adjacent to the dorsal kidney and spleen are also observed. Internal gross symptoms include a pale liver, kidney and spleen.

Microscopic examination of viscera shows extensive degeneration and necrosis in kidney, spleen and pancreas tissues. The most severe damage occurs in the hematopoietic tissues of the kidney and spleen (Amend et al. 1969).

Outbreaks of disease in hatcheries are characterized by their explosive nature and high mortality rates. Natural epizootics have occurred in juvenile rainbow and steelhead trout as well as sockeye salmon. By experimentation, cutthroat trout *(Salmo clarki)* have been found susceptible. Coho salmon *(Oncorhynchus kisutch)* are resistant.

Annual epizootics in juvenile chinook salmon have occurred at the Coleman National Fish Hatchery and the Nimbus Hatchery, both in California, for many years (Ross et al. 1960). The disease was originally referred to as the Sacramento River chinook disease and the causative agent has been shown to be a bullet shaped virus almost identical to that causing IHN infection in other salmonids. Laboratory studies have revealed a slight difference, which may be either antigenic or due to a greater stability of the infectious property of the chinook strain (McCain et al. 1971). The disease's symptoms in chinook salmon are similar to those in sockeye salmon and rainbow trout infected with IHN, and the hematopoietic tissue of the kidney is the site of the most extensive damage (Yasutake et al. 1965).

INFECTIOUS PANCREATIC NECROSIS

Historical Review

The disease now known as infectious pancreatic necrosis was first described by McGonigle (1941) as an "acute catarrhal enteritis" in very young brook trout *(Salvelinus fontinalis)*. Two of the characteristic symptoms of this disease are violent whirling and horizontal corkscrewing, accompanied by a high mortality rate.

The next reported outbreak of the disease occurred in juvenile brook trout at the hatchery of the Eastern Fish Disease Laboratory at Leetown, West Virginia in 1953 (Snieszko et al. 1959). To determine if the disease was infectious in nature, these investigators held four lots of eyed brook trout eggs from different hatcheries for observation. Pancreatic necrosis (IPN) appeared spontaneously in trout from one hatchery. Those from the other sources were

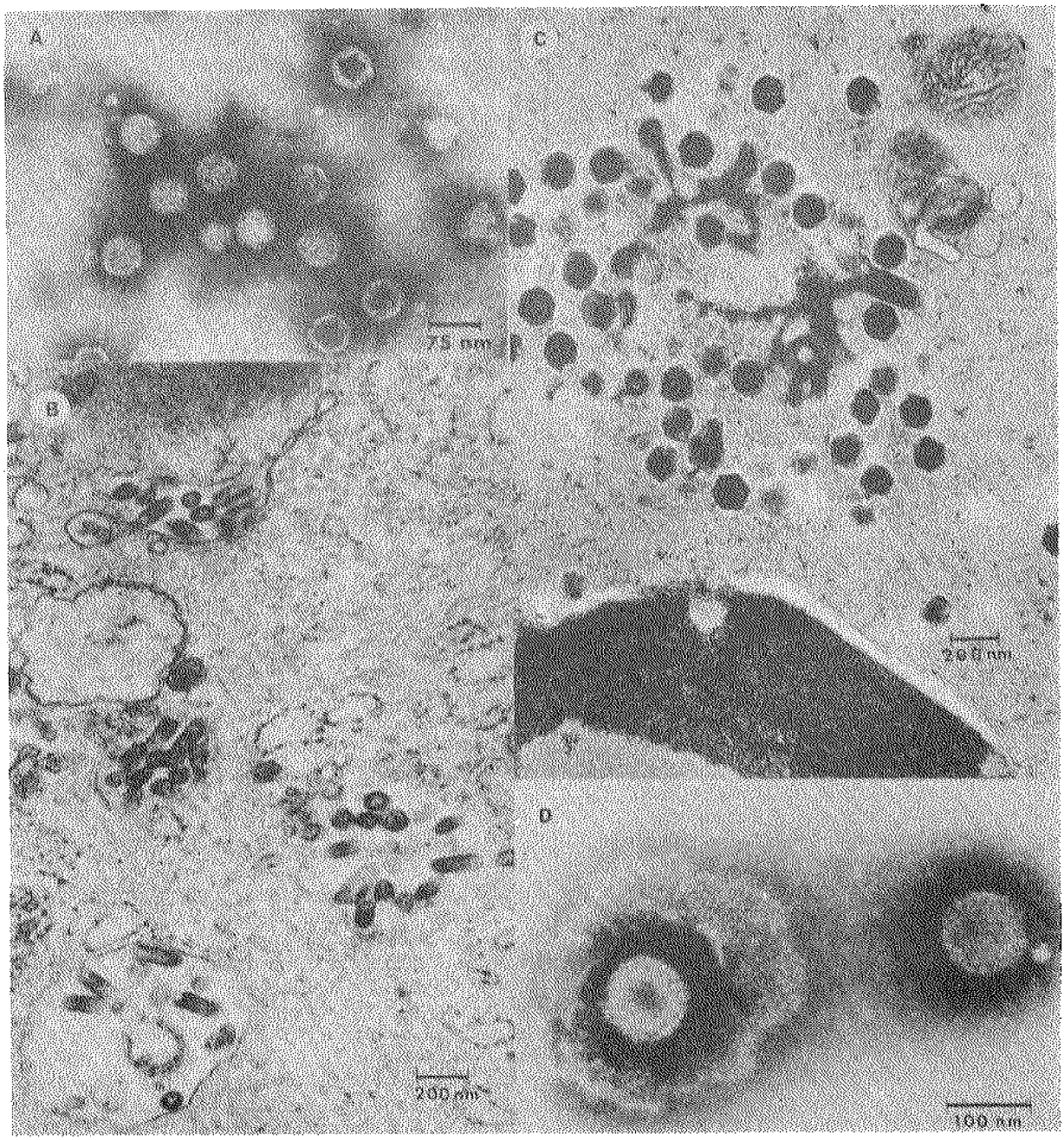

FIGURE 1. Electron micrographs of 4 viruses, causing disease in certain salmonid fish.

A. Virions of infectious pancreatic necrosis (IPN).

B. Virions of infectious hematopoietic necrosis (IHN).

C. Virions of viral erythrocytic necrosis (VEN) in cytoplasm of chum salmon erythrocytes.

D. Virions of *Herpesvirus salmonis*.

exposed to the infection by adding to the water aqueous suspensions of homogenates prepared from entire trout showing symptoms of the disease. In all cases typical IPN developed in fish exposed to infection, while control fish remained healthy. The infectious nature of the disease was thus established. Outbreaks of what was apparently the same disease among juvenile brown trout *(Salmo trutta)* and Atlantic salmon *(Salmo salar)* were reported from other hatcheries. A viral etiology was suspected.

The virus was first isolated by Wolf et al. (1960) from bacteria free filtrates of the tissues of moribund infected brook trout. The filtrates were inoculated in tissue cultures prepared from brook trout tissues. A cytopathic effect (CPE) was produced by the virus, which was carried through nine serial passages. Culture fluids from the sixth and eighth passage produced the typical disease when fed to susceptible young brook trout.

The virus causing IPN was detected for the first time in Oregon's salmonids during 1971. An epizootic of infectious hematopoietic necrosis was in progress among rainbow trout in a small private lake. Cutthroat trout were resident in a creek draining this lake, and 11 of these adult fish were analyzed in the laboratory to determine if the IHN infection had spread to them. Surprisingly, this analysis revealed the presence of IPN virus rather than IHN virus. These fish were asymptomatic, and served as IPN carriers. During the same year IPN virus was isolated from adult coho salmon being spawned at the Bonneville Hatchery on the Columbia River (McMichael 1974). These salmon also appeared to be healthy carriers of the virus.

Characteristics of the Virus

Since this virus was first isolated by Wolf in 1960, numerous additional isolations have been made from infected salmonids by a number of investigators in North America, Europe and Japan. Early reports on the size and morphology of the virus were found to be in error, and it is now established that the virions are icosahedral with a mean diameter of 55 nm and a capsomere count of 92. The electron microscope studies of Moss and Gravell (1969) and Lightner and Post (1969) agree on these characteristics. The viral genome is composed of ribonucleic acid (RNA), but the question of its single or double stranded nature has been controversial. Kelly and Loh (1972), on the basis of polyacrylamide gel electrophoresis, concluded that the RNA was a single non-segmented component with a molecular weight of 3.2×10^6. Its susceptibility to ribonuclease, base composition and resistance to thermal denaturation all appeared to confirm its single stranded nature. Nicholson (1971) also concluded the RNA was single stranded based on studies with fluorescence microscopy and susceptibility to RNAse. On the other hand, Argot (1969) reported the appearance of pale green cytoplasmic inclusions in infected tissue culture cells stained with acridine orange about six hours after infection, indicating the presence of double stranded RNA. Furthermore she observed a thermal denaturation curve for the viral RNA showing a sharp deflection characteristic of double stranded nucleic acids.

The most recent report on the subject is that of Dobos (1976). He used high resolution polyacrylamide gel electrophoresis followed by autoradiography, which indicated that the virus contains two segments of double stranded RNA with M.W.s of 2.5×10^6 and 2.3×10^6. He found the RNA resistant to ribonuclease. The virus has the same size and shape, as those of the reovirus group, but it lacks the inner capsid found in members of this group. Also its double stranded RNA has only two segments instead of 10 as reported for the reoviruses. The morphology of the virions is shown in the electron micrograph of Figure 1a.

Pathology

The first recognized epizootic of infectious pancreatic necrosis was reported in fingerling brook trout by Snieszko et al. (1959). Since that time outbreaks have been described in rainbow, cutthroat, Atlantic salmon and brown trout. A sudden increase in mortality is often the earliest sign of an IPN epizootic (Yasutake 1970). Many of the infected fish exhibit an abnormal type of swimming, characterized by a corkscrewing or whirling motion. They may also show one or more of the following signs: dark coloration, exophthalmia or ascites. Internally, a mucoid plug in the stomach and anterior intestine is often present. A pale liver and spleen, petechiae in the caecal area, and an empty digestive tract are also characteristic.

Histologically, there is massive necrosis of the acinar cells of the pancreas, with pyknosis of nuclei and inclusions in the cytoplasm. Islet tissue may also show such changes. Pathological changes in the hematopoietic tissue of the kidney have been found frequently in terminal cases, which resemble those found in early cases

of infectious hematopoietic necrosis (Yasutake et al. 1965).

It has been shown that healthy adult brook trout can be infected without showing evidence of disease (Wolf and Quimby 1969). However, the infected fish actively shed the virus in the feces, although it may in time be cleared from the digestive tract. It appears highly probable that when epizootics occur among very young fish some of the survivors will become carriers and may continue to shed virus indefinitely (Billi and Wolf 1969).

VIRAL ERYTHROCYTIC NECROSIS (VEN)[1]

Historical Review

Laird et al. (1969) described a pathological condition in the erythrocytes of cod *(Gadus morhua)*, seasnail *(Liparis atlanticus)* and shorthorn sculpin *(Myxocephalus scorpius)* collected from coastal waters of eastern Canada and the northeastern United States. By light microscopy, they observed eosinophilic inclusion bodies measuring up to 1 μm in diameter in the cytoplasm of infected erythrocytes. The nuclei of such cells became distorted, and a round clear vesicle developed within them that contained dense staining particles from 0.25 to 0.5 μm in diameter. They suggested a possible viral etiology and named the disease piscine erythrocytic necrosis.

Walker (1971) found what appeared to be the same condition in two of 18 cod from waters off the coast of New Brunswick. Ten percent or more of erythrocytes were affected. An electron microscopic examination of these cells revealed the presence of large cytoplasmic particles with hexagonal profiles, similar in appearance to virions of the iridovirus group such as lymphocystis virus of fish. In groups containing 20 to 50 virions, the center spacing was about 350 nm. Cells containing these particles also exhibited nuclear degeneration.

Sherburne (1973) observed the same pathological changes in erythrocytes of the Atlantic herring *(Clupea harengus harengus)* and noted that the condition was associated with water temperatures of 13.8°C or above.

Johnston and Davies (1973) described a similar condition in erythrocytes of the blenny *(Blennius gattis)* taken off the coast of Aberystwith, Wales. Using electron microscopy they found the cytoplasmic bodies with hexagonal or pentagonal profiles measuring 200 to 300 nm in diameter from opposite apices.

Recent work by Sherburne (1977) disclosed the presence of the same type of inclusions in erythrocytes of the anadromous alewife, *Alosa pseudoharengus*, from coastal streams in Maine. Among prespawning fish, 56.1 percent of 661 specimens were affected compared with 10.5 percent of 57 postspawning specimens.

The same or a very similar disease in Atlantic herring was reported by Philippon et al. (1977) and Reno et al. (1978). Eleven percent of the fish examined showed the erythrocytic pathology when first examined, but 13 days after capture 95 percent of them were affected. The cytoplasmic particles with hexagonal profile were much smaller than those in cod, and measured only 146 nm in diameter with a dense center 96 nm in diameter.

Characteristics of the Virus

More recent studies of VEN in the blood of Atlantic cod have been reported by Appy et al. (1976) and Walker et al. (1977). Both groups found the hexagonal particles averaging about 330 nm in diameter. The particles contained an outer electron dense layer about 35 nm wide, a medium electron translucent layer about 16 nm wide and a central electron dense core about 230 nm in diameter. Walker et al. (1977) indicated that a cytoplasmic structure adjacent to a group of the virions, which they called the viroplasm, was Feulgen positive. Because of this, they concluded that the hexagonal particles represented a DNA virus. However, this evidence must be considered presumptive.

Evelyn and Traxler (1978) found VEN in both chum salmon *(Oncorhynchus keta)* and pink salmon *(O. gorbuscha)* taken off the Pacific coast of North America in 1976. It was frequently encountered among individuals of these species held in net pens in seawater, and seemed to be most severe during the summer. Mortality rates were low (i.e. up to 0.3 percent).

These workers carried out transmission experiments using tissue extract prepared by homogenizing infected chum salmon kidney and spleen, and passing the centrifuged extract through a 0.45 μm membrane filter.

[1] Originally called piscine erythrocytic necrosis, or PEN.

The filtrate was then inoculated intraperitoneally in chum or pink salmon. Controls received injections of buffered saline. Three serial passages were made in chum salmon and a fourth in pink salmon.

Nine of 10 pink salmon inoculated with fourth passage material showed infection within three weeks as determined by the examination of blood cells. The same inoculum failed to infect chinook, coho or sockeye salmon. Attempts to isolate the virus by inoculating RTG-2 (rainbow trout gonad cells), CHSE-214 (chinook salmon embryo cells) and fathead minnow cell cultures were unsuccessful. The tissue filtrate heated at 60°C for 15 minutes was no longer infectious for chum or pink salmon. The average measurement between opposing apices of the hexagonal and pentagonal particles in the blood of experimentally infected fish was 190 nm. The outer electron dense coat was about 8 nm wide and surrounded an electron lucent zone 22 nm wide. A central circular to hexagonal core or nucleoid was 130 nm in diameter. Thus, the particle size was smaller than that reported for virions in the blenny and cod, but larger than those in the Atlantic herring. Inclusions in erythrocytes were Feulgen positive. Preliminary evidence was obtained indicating that Pacific herring *(Clupea harengus pallasi)* also suffer from this disease, and that tissue filtrates from infected herring can induce the disease in chum and pink salmon.

The morphology of the virions in chum salmon erythrocytes is shown in the electron micrograph of Figure 1c.

The first report apparently indicating replication of a virus in VEN infected erythrocytes in an *in vitro* system was made by Reno and Nicholson (1978). Infected and uninfected cod erythrocytes were suspended in buffered saline containing an increased salt concentration and incubated at 4, 8 or 15°C. Synthesis of protein and nucleic acids was measured by incorporation of radioactive precursors. The cumulative incorporation of ^{3}H-amino acids was greater in infected cells by the sixth day indicating an increased protein synthesis compared with uninfected control cells. DNA synthesis was greater in infected cells initially with a steady increase in cumulative incorporation of ^{3}H-thymidine, which reached a maximum on the sixth day. Typical VEN virions were found in infected cells. The greater incorporation of precursors into protein and DNA in infected cells compared with controls is presumed to be the result of viral replication.

Pathology

The chief pathological change observed in VEN infected fish has been the abnormal erythrocytes, which contain eosinophilic inclusion bodies and distorted nuclei. The latter may contain a round clear vesicle enclosing a number of dense staining particles from 0.25 to 0.5 µm in diameter.

Evelyn and Traxler (1978) found that infected chum and pink salmon were anemic, and histologically the kidney showed extremely active hematopoiesis.

HERPESVIRUS SALMONIS

A fourth virus infecting a salmonid species has recently been discovered in the Pacific Northwest. It was isolated by W. G. Taylor (U.S. Fish and Wildlife Service) in 1975 from postspawning rainbow trout brood stock at the Winthrop National Fish Hatchery in Washington and characterized by Wolf et al. (1978). It was found to be a member of the herpesvirus group, and its optimum temperature for replication in the RTG-2 cell line was 5 to 10°C. The morphology of the virions is shown in Figure 1d. The brood stock from which it was isolated had a three-year history of higher than normal postspawning mortality. Wolf et al. (1978) reported that the original infected stock was taken out of production, and that since August 1976, all virological examinations of Winthrop strain fish stocks have utilized cultures of RTG-2 cells incubated at 10°C. However, no additional isolations of the virus have been made. They speculated that the agent is probably not native to North America and may have been introduced with an unauthorized importation of a species of salmonid from Japan.

Beginning in 1977, the procedure used to detect viruses in Oregon's salmonids was modified to make it more favorable for the isolation of *Herpesvirus salmonis*. The cultures of CHSE-214 (chinook salmon embryo cells) and STE-137 (steelhead trout embryo cells), inoculated with the prepared samples to be tested, are now incubated at both 10 and 15°C. Only the lower temperature is in the optimum range for replication of this virus. However the agent has never been detected in any viscera or ovarian fluid sample from salmonids in Oregon.

METHODS OF DETECTING IPN AND VEN VIRUSES

Collection of Samples

Whenever possible, the number of fish

taken for virus examination from a population was determined according to Ossiander and Wedemeyer (1973). Generally, 60 fish were taken from large populations to allow for the detection of a minimum 5 percent carrier incidence with a 95 percent confidence level, assuming randomness. When 60 fish could not be obtained the number available was examined.

The type of sample taken depended on the size and sexual maturity of the fish, and the type of virus suspected to be present. Standard methods (Fish Health Section of the American Fisheries Society 1975) for collecting tissue samples were followed. Three types of samples were obtained: viscera, gonadal fluids and whole fish. Tissues were generally collected as pools of organs or fluids from five fish resulting in 12 pools from a 60-fish test lot.

Juvenile fish 4-10 cm long were eviscerated and the removed viscera, including kidney, were processed. Early in the study, kidney, liver and spleen were taken from fish longer than 10 cm. Fish of this size were considered as adults for sampling purposes as they were larger than the usual size of fish susceptible to the acute phase of the virus diseases. The liver was eliminated later (Yamamoto 1974).

Ovarian fluids were collected whenever possible and were the sample of choice when examining adult fish for IHN virus in the carrier state.

Whole fish samples were also used to detect viruses. These preparations were composed of alevins and juveniles up to 4 cm long. In this case, the number of fish per pool was increased from five to 10.

Organs, fluid and fish were transported to the laboratory on crushed ice. No transport medium was added. Tissue samples were stored at 4°C (never frozen) until prepared for inoculation on to cell cultures. All samples were processed within one week after arriving at the laboratory.

Examination of Samples

Two autonomous salmonid cell cultures, chinook salmon embryo (CHSE-214) and steelhead trout embryo (STE-137), were used for virus examination of all samples (Fryer 1965). The culture vessels were plexiglass tissue culture plates having 96 flat bottom wells. Throughout most of this work, Eagle's Minimum Essential Medium with fetal calf serum was used and is the preferred medium for cultivating these cells. This preparation is designated MEM and the percent sign following indicates the amount of fetal calf serum contained (Eagle 1959). The cell cultures were seeded into the wells where they formed monolayers during a minimum incubation period of 24 hours. No cell cultures older than four days were used for virus isolation. Prior to inoculation with the sample the culture medium was removed from the wells and replaced with one drop per well of fresh medium, MEM-5 percent. After inoculation with the sample to be tested the culture plates were sealed with a mylar cover and incubated at 18°C for a minimum of 14 days. The cells in each well were examined microscopically at 24-hour intervals for the development of cytopathic effects (CPE) indicative of the presence of a virus.

Ovarian fluid samples were diluted 1:5 (v/v) with buffered saline and filtered through a 0.45 μm membrane filter in a Swinney holder (Millipore Corp.). One drop of filtered fluid was placed in each of six wells containing monolayers of cells. The final dilution of the fluid was 1:10.

Sample pools of viscera or whole fish were weighed and diluted 1:10 (w/v) in buffered saline. Each sample was homogenized for two minutes with a Virtis Model 23 Macro Homogenizer at 23,000 rpm. The homogenate was centrifuged at 3,000 x g at 5°C for 20 minutes. One ml of the supernatant was diluted with 4 ml of an antibiotic solution containing 10,000 IU/ml of penicillin, 10,000 μg/ml of streptomycin sulfate and 10,000 IU/ml of nystatin. The mixture was allowed to stand at room temperature for two to six hours or at 4°C overnight. Next, one ml of the mixture was filtered through a 45 μm membrane filter and one drop added to each of six wells per culture plate. The final dilutions of the visceral and whole fish samples were 1:100.

Isolation of Viruses

Samples were considered negative after 14 days of incubation if no CPE was seen by microscopic examination of inoculated cell monolayers. If CPE was observed, 0.1 ml of fluid was removed from the well and diluted 1:100 (v/v) in buffered saline. New plate cultures were inoculated with this diluted fluid. The latter then represented a total dilution from the original sample of 1:10,000. If CPE was again seen within 14 days on the subculture plate a further 1:100 (v/v) dilution of the culture fluid was made and new cell cultures inoculated. The fluid used to inoculate these cultures now represented

a 1:1,000,000 dilution of the original sample. The subcultures served to eliminate by dilution the toxicity present in some samples. Furthermore, viruses, if present, would replicate at each subculture, and thus would still be present in the highly diluted culture fluids. The appearance of typical CPE in the cells of the final subculture was presumptive evidence of the presence of a virus.

Identification of Isolated Viruses by Serum Neutralization

Fish viruses are identified by demonstrating the ability of a known immune serum specific for one particular virus, prepared in a rabbit injected with that agent, to neutralize the infective capacity of an unknown virus (i.e. to prevent the development of CPE in cell cultures). If the unknown is neutralized by this serum it is thus identified as the same virus as that for which the serum is specific.

Accordingly, when CPE appeared in the cells of the final subculture, a serum neutralization test in plate cultures was done to confirm the identity of the virus. Both IPN and IHN viruses could be provisionally distinguished by the type of CPE they produced, and the type of antiserum and control (known) virus used in the neutralization test was chosen on this basis. Figure 2 shows normal cultures of CHSE-214 and STE-137 cells and cultures exhibiting CPE characteristic of IPN and IHN viruses.

From the final subculture, 0.1 ml of fluid was withdrawn from wells showing CPE and tenfold dilutions made in buffered saline. The known or control virus was similarly diluted. One-half ml of each dilution of both the unknown and control viruses was transferred to sterile test tubes. Antiserum, prepared in our laboratory, was diluted to 1:100 with buffered saline. One-half ml of the diluted neutralizing antiserum was added to each of the tubes containing the diluted unknown and the control virus. The mixtures were shaken and incubated at 18°C for one hour with agitation at 15-minute intervals.

Next a second culture plate was obtained which had been seeded with homologous cells the previous day and incubated for 24 hours. The old culture medium was removed and two drops per well of fresh medium, MEM-10 percent, were added to 24 of the 96 wells. These served as medium controls and to separate the known virus control from the unknown virus. One drop of each dilution of the unknown virus was added to a group of four wells, requiring 16 wells in all for four dilutions. Similarly four dilutions of the control virus required another 16 wells. These two groups of 16 cultures served as positive controls containing the same dilution of the viruses as the antiserum virus mixtures.

Two other groups of 16 wells received the antiserum virus mixtures. Two drops of each dilution of the unknown virus plus antiserum were added to each of four wells, requiring 16 wells in all for the four virus dilutions. In like manner the mixtures of control virus dilutions with antiserum required another 16 wells.

The remaining eight wells on the culture plate each received one drop of buffered saline and one drop of the antiserum dilution used in the mixtures, and served to determine if the serum alone could kill the cells.

The culture plate for the neutralization test was then sealed and incubated at 18°C for 72 hours for IPN tests and 120 hours for IHN tests. If CPE appeared in the positive control wells of both the known and unknown viruses, but not in the antiserum virus mixture wells of both, the unknown virus was identical to the known control virus and was thus identified.

If the known control virus, but not the unknown virus, was neutralized by the antiserum another neutralization test was done using antiserum against another fish virus. All viral agents isolated were neutralized by antiserum against IPN (strain CTT-IPN) or IHN (strain Nan-S-IHN) viruses.

isolations in Oregon

The viral exanimations discussed in this report began in 1958 and were made at irregular intervals until 1971. From 1971 through 1978 a regular and extensive survey was conducted. During this period both adult fish and juveniles, or alevins, from most of the hatcheries operated by the Oregon Department of Fish and Wildlife, as well as wild fish from numerous lakes and streams, were sampled extensively. They were examined in the fish virology laboratory, Department of Microbiology, Oregon State University for the presence of fish viruses and in certain cases for specific antibody against these agents.

Figure 3 shows all of the locations in eastern and western Oregon where fish samples were collected for examination.

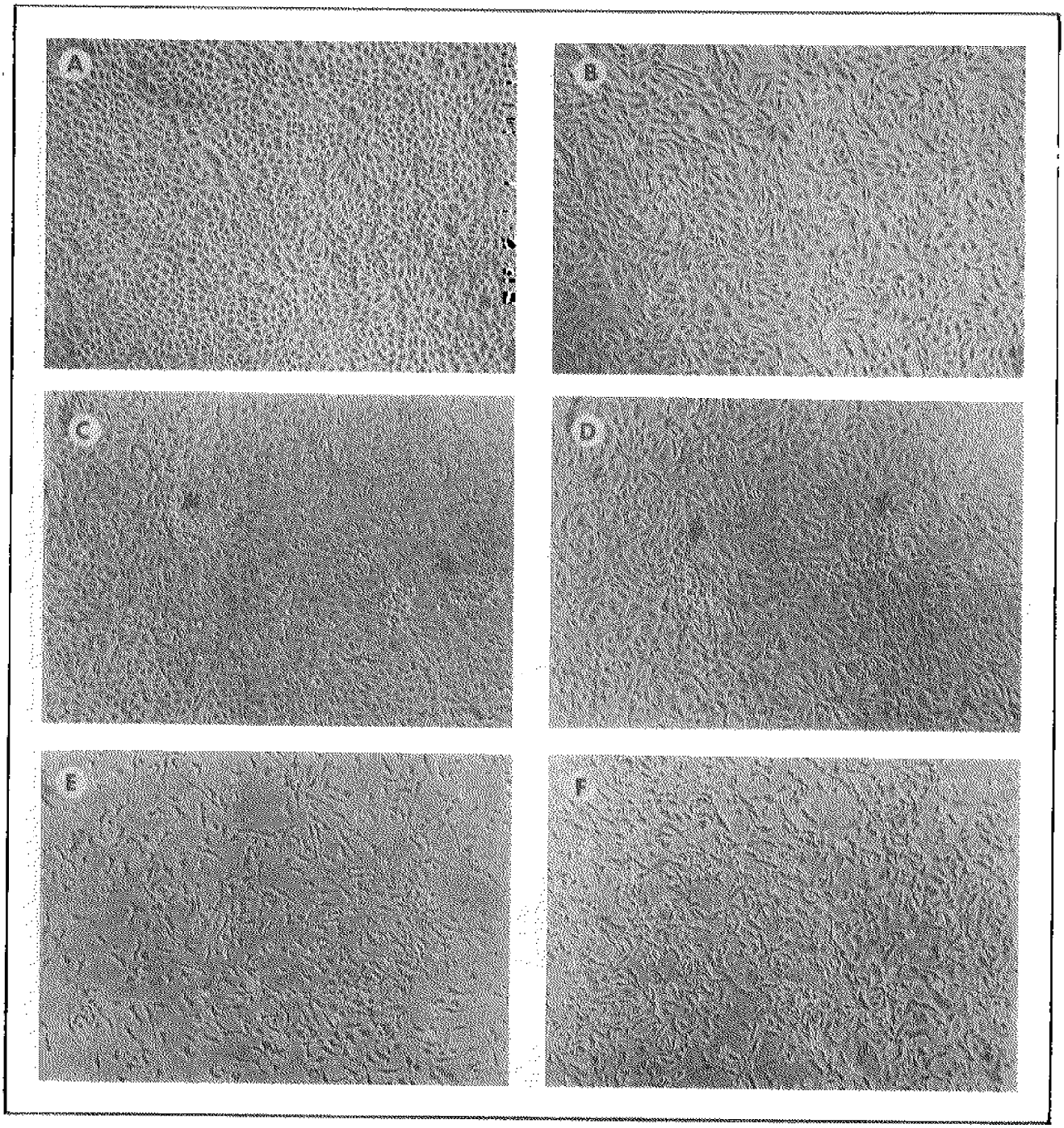

FIGURE 2. Phase contrast photomicrographs of monolayer cultures of uninfected and virus infected salmonid cells. Uninfected (A) CHSE-214, and (B) STE-137 cells. IHN virus infected (C) CHSE-214, and (D) STE-137 cells; arrows indicate cells showing virus induced cytopathic effects (CPE). IPN virus infected (E) CHSE-214 and (F) STE-137 cells; CPE is extensive and involves the entire cell population. Magnification is 300 x.

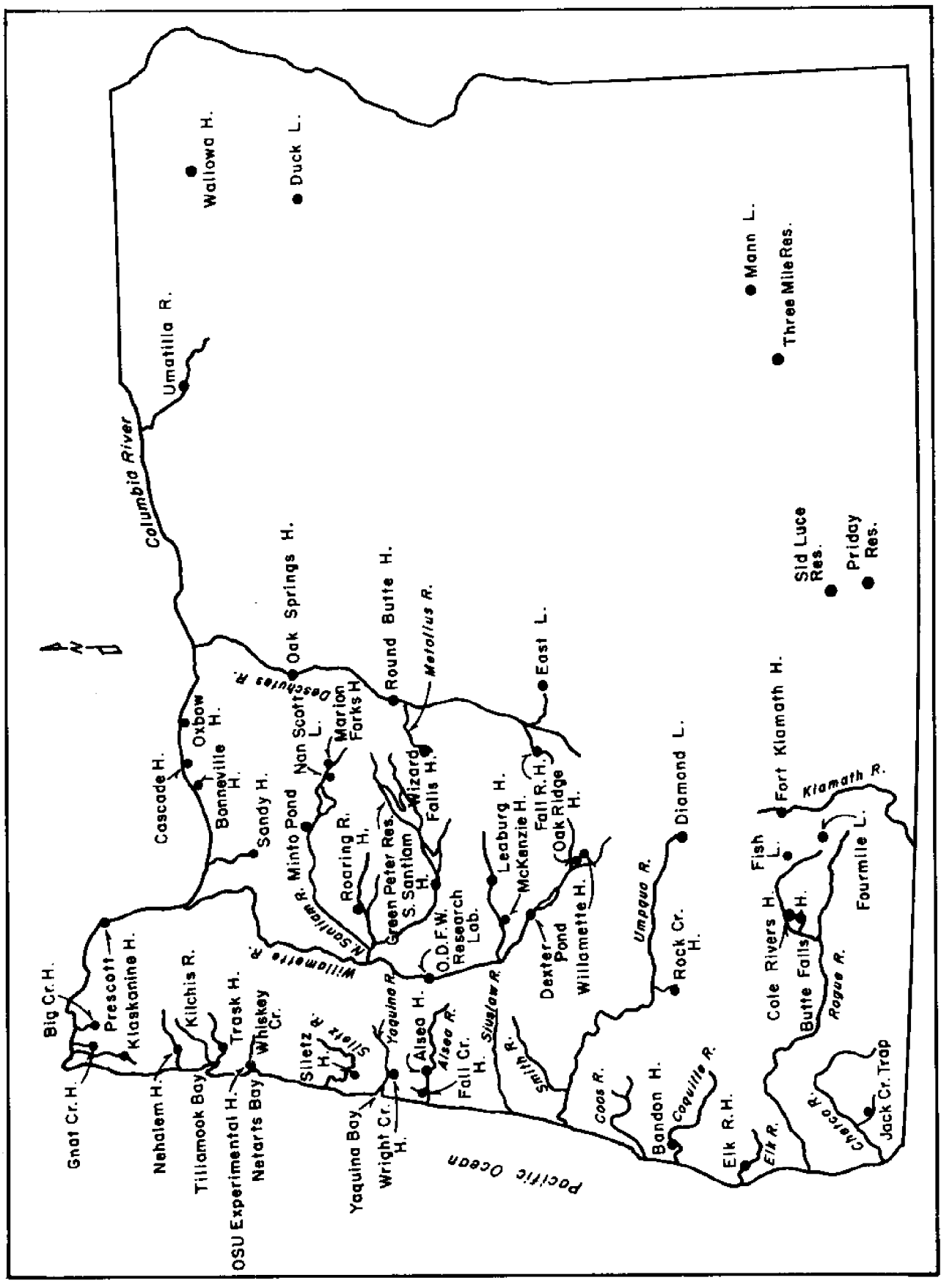

FIGURE 3. Waters in eastern and western Oregon where salmonid populations were sampled and analyzed for the presence of IHN or IPN virus; and coastal waters where certain species were examined for VEN.

In most cases samples from these sites were collected at several different times. In addition this map also indicates many of the hatcheries in the Willamette Valley and a few lakes or streams in central Oregon where salmonid populations were studied. However, because of the large number of collection sites from which fish were examined in these two areas, and because the majority of virus isolations came from fish samples taken in these areas, the locations of all hatcheries, lakes and streams where fish were collected in central Oregon and the Willamette Valley are shown in Figure 4. Figure 5 indicates the hatcheries and other waters where either IHN or IPN virus, or both, were isolated, and also the coastal waters where VEN inclusions were found in the erythrocytes of chum salmon *(O. keta)* or Pacific herring *(Clupea harengus pallasi)*.

Tables 1 and 2 contain summarized information concerning all isolations of IHN and IPN viruses in Oregon hatcheries, lakes and rivers, and also information on the detection of VEN virus infection by examination of erythrocytes. Table 3 shows the losses of hatchery fish resulting from infection with IPN or IHN virus where this information is available. Tables 4-8 (Appendix) give the pertinent information concerning the collection and analysis of each sample of salmonids examined.

In addition to the Oregon Department of Fish and Wildlife hatcheries, salmonid populations from the following private hatcheries were examined on one or more occasions for the presence of fish viruses: Anadromous, Inc.; Oregon Aqua-Foods; Roggo Hatchery; Domsea Farms, Inc.; Thom Hatchery; Brian Hatchery; Trillium Lake Trout Farm; and Mt. Hood Community College. Altogether at least 30 samples from these sources were analyzed, but no virus was detected in any of them.

ISOLATIONS OF THE VIRUS OF INFECTIOUS HEMATOPOIETIC NECROSIS (IHN) IN OREGON

During the 20-year period covered by this report, salmonid populations in all Oregon hatcheries have been examined for the presence of fish viruses. In most cases samples of both adult fish and juveniles or alevins have been studied. Table 5 gives the data for those hatcheries in the Willamette River drainage. It may be noted that the IHN virus has never been detected in any of these hatcheries since 1959. It has never been found in hatcheries along the Columbia River (Table 4), and has been detected in only two of those in the Deschutes River drainage (Table 7). Figures 3-5 give the locations of all hatcheries.

The virus was not found again in any Oregon hatchery between 1959 and 1975 (Table 7). In March, 1975, juvenile rainbow trout and kokanee salmon began dying in large numbers at Wizard Falls Hatchery on the Metolius River. The trout were hatched from eggs taken from the Roaring River Hatchery broodstocks, and the kokanee salmon were spawned from feral fish in either North Twin or Suttle Lake. A virus etiology for the epizootic was suspected because of the signs, which included darkening of the dorsal surface, trailing fecal casts, exophthalmia and ascites.

Laboratory analysis of large samples of rainbow trout juveniles taken on three separate dates in March and one in April revealed the presence of IHN virus, and the typical pathology observed provided confirming evidence that this was the cause of death. However, the first tests seemed to indicate that both IHN and IPN viruses were present in the rainbow trout. Extensive analysis confirmed the presence of both agents, and further revealed that both viruses could be detected in the same fish (Mulcahy and Fryer 1976). Again, the pathology indicated that IHN was probably the most important cause of mortality.

A population of about 150,000 juvenile Atlantic salmon located within the hatchery building showed no signs of infection during this period.

The source of the IHN virus in this epizootic is unclear. The kokanee salmon eggs had been taken from two sources, North Twin and Suttle Lakes. Suttle Lake had been used repeatedly in the past as a source of kokanee eggs and no unusual losses had been noted. A relatively small number of kokanee from North Twin Lake were tested for the virus with negative results, but only visceral samples were available. Conceivably the presence of the virus in carrier fish could have been missed with no ovarian fluid for examination.

It was first thought that the source of the IPN virus was the broodstock from Roaring River Hatchery, but examination of groups of rainbow juveniles hatched from Roaring River eggs at other hatcheries failed to isolate any virus. The most likely source of infection was then assumed to be virus at Wizard Falls Hatchery that had survived in the disinfection procedures instituted after an IPN outbreak there in 1974.

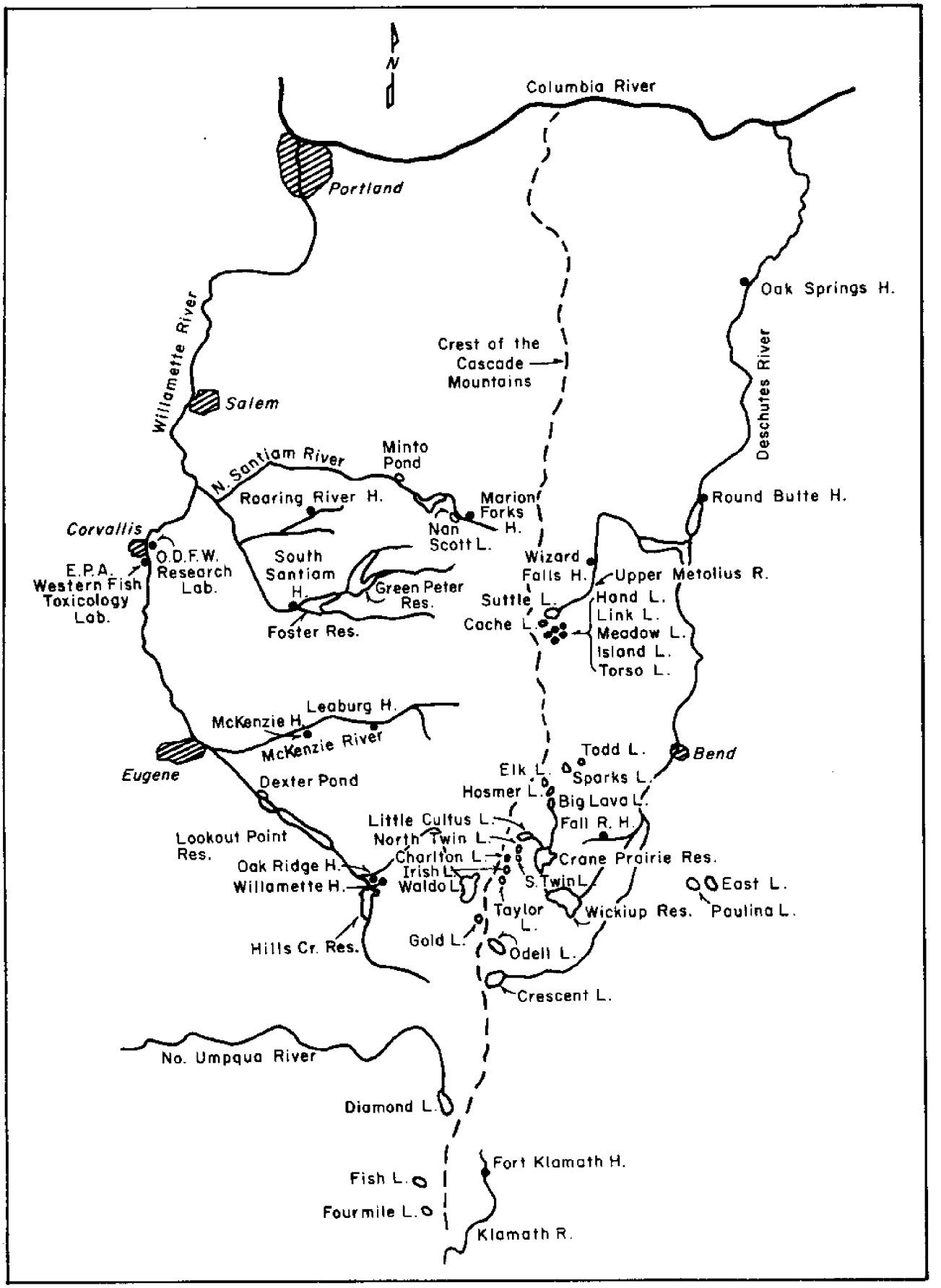

FIGURE 4. Waters in central Oregon and the Willamette valley where salmonid populations were sampled and analyzed for the presence of IHN or IPN virus.

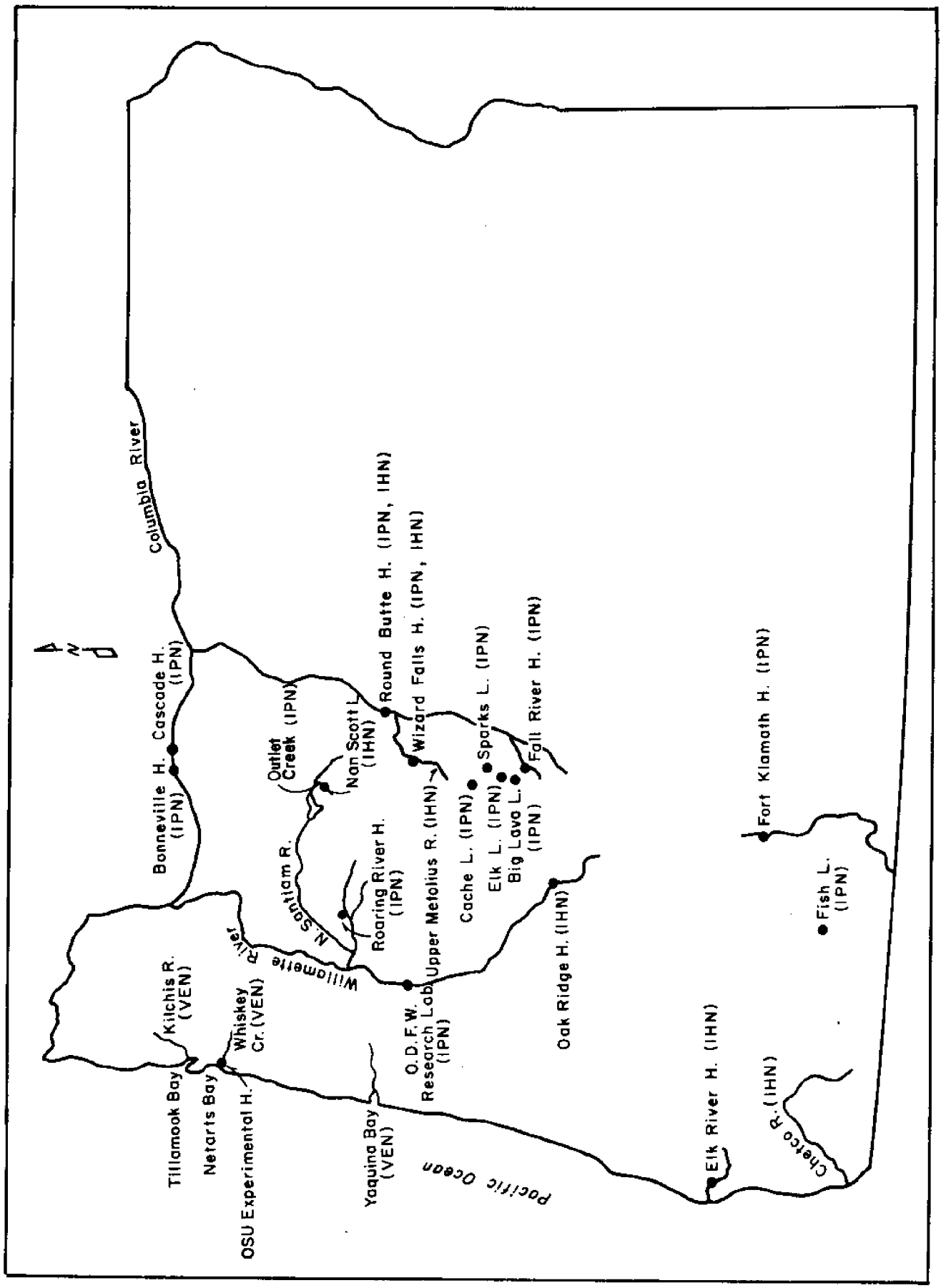

FIGURE 5. Location of hatcheries, lakes and streams whre IHN or IPN virus or both were isolated; and coastal waters where VEN virus was found.

Table 1. Summarized data on Oregon fish hatcheries where IHN or IPN viruses have been isolated.

Hatchery	Species and stage of development		Date of first isolation	Virus	Fish mortality caused by virus
Oakridge	SS[A]	Juv[B]	9/58	IHN	Yes
Bonneville	Co	Ad	10/71	IPN	No
	Co	Al	2/72	IPN	No
Cascade	Co	Juv[1]	2/72	IPN	No
	Co	Ad	10/72	IPN	No
Round Butte	ChS	Ad	8/73	IHN	No
	ChS	Ad	9/73	IPN	No
	St	Juv	4/75	IHN	Yes
	St	Ad	1/76	IHN	No
Fall River	BT	Juv	6/73	IPN	Yes
	Rb	Juv	6/73	IPN	Yes
Roaring River	Rb	Ad Br	12/73	IPN	No
Oregon Dept. of Fish and Wildlife Research Lab., Corvallis	ChS	Juv	12/73	IPN	No
Fort Klamath	Br	Juv	1/74	IPN	No

Table 1 (continued)

Hatchery	Species and stage of development		Date of first isolation	Virus	Fish mortality caused by virus
Wizard Falls	BT	Juv	1/74	IPN	Yes
	K	Juv	3/75	IHN	Yes
	Rb	Juv	3/75	IPN/IHN	Yes
Elk River	ChF	Ad	1/76	IHN	No
	ChF	Juv	4/76	IHN	Yes

[1]Fish were from Bonneville Hatchery Stock.

A. Abbreviations for fish species.

Br	brown trout
BT	brook trout
Ct	cutthroat trout
Ct Lah	Lahontan cutthroat trout
K	kokanee salmon
Rb	rainbow trout
AS	Atlantic salmon
RB-Red	red banded rainbow trout
ChF	fall chinook salmon
ChS	spring chinook salmon
Co	coho salmon
ST	steelhead trout
StS	summer steelhead trout
StW	winter steelhead trout
SS	sockeye salmon
ChuS	chum salmon
PaH	Pacific herring

B. Abbreviations for stage of development.

Al	Alevin
Juv	Juveniles
Ad	Adult

Table 2. Summarized data on Oregon lakes and rivers where IHN or IPN viruses have been isolated from salmonid fish; and on coastal waters where VEN virus inclusions have been found in fish erythrocytes.

Location	Species	Date of first isolation or detection	Virus
Nan-Scott Lake	Rb Ad (A,B)	8/71	IHN
Outlet Creek for Nan-Scott Lake	Ct Ad	9/71	IPN
Fall River	BT Ad	6/73	IPN
Fish Lake	BT Ad	9/73	IPN
Big Lava Lake	BT Ad	9/73	IPN
Elk Lake	BT Ad	9/73	IPN
Cache Lake	BT Ad	7/74	IPN
Sparks Lake	BT Ad	7/74	IPN
Upper Metolius River	K Ad[1]	10/75	IHN
Elk River	ChF Ad	1/76	IHN

Table 2 (continued)

Location	Species	Date of first isolation or detection	Virus
Chetco River	ChF Ad	12/77	IHN
OSU Experimental Hatchery at Netarts Bay	ChuS Ad	11/76	VEN
Kilchis River	ChuS Ad	12/76	VEN
Yaquina Bay	PaH Ad	3/77	VEN

1. Landlocked Oncorhynchus nerka.

A. Abbreviations are defined in Table 1
B. Abbreviations are defined in Table 1

Table 3. Summary of Oregon hatchery fish killed by viral disease or destroyed to prevent spread of fish virus infection.

Hatchery	Year	Virus	Species (A,B)	Number of Fish Lost
Oakridge	1958	IHN	SS Juv	240,000[3]
Wizard Falls	1974	IPN	BT Juv	693,000
	1975	IHN	K Juv	600,000
		IHN/IPN	Rb Juv	3,700,000
		(none)[1]	AS Juv	150,000
Fort Klamath	1974	IPN	Br Juv	70,000
Round Butte	1975	IHN	St Juv and Al	500,000
	1976	IHN	St Juv	222,000
	1978	IHN	St Juv and Al	361,000
Fall River	1973	IPN	BT Juv	1,513,000
		IPN	Rb Ad (legal size)	71,000
		IPN	Rb Juv	577,000
Elk River	1976	IHN	ChF Juv and Al	42,250
	1976	IHN[2]	ChF Al and Juv	8,900
	1978	IHN[2]	ChF Al and Juv	34,000

[1]Fish were destroyed as a precautionary measure

[2]Fish from the Chetco River, held at the Elk River hatchery, were included

[3]Fish were also infected with *Renibacterium salmoninarum*, which is the causative agent of bacterial kidney disease. Records are incomplete and the amount of fish lost was estimated.

A. Abbreviations are defined in Table 1.
B. Abbreviations are defined in Table 1.

In April, 1975, about a month after the Wizard Falls epizootic, steelhead trout juveniles at the Round Butte Hatchery began dying in large numbers. When a search for possible bacterial or protozoal pathogens gave negative results, tests for the presence of known salmonid viruses were begun. Three large samples of these fish were collected at different times during late April and early May, and in all three cases IHN virus was isolated and positively identified (Table 7). The infected fish were confined to three large tanks that were somewhat isolated from the rest of the raceways containing fish. Juvenile steelhead located within the hatchery building were not affected by the disease, nor were spring chinook salmon juveniles in the raceways. No virus was detected in steelhead smolts that were scheduled for release.

In an effort to determine the source of the virus causing this epizootic, chinook salmon adults being spawned in August, 1975 at Round Butte Hatchery were examined. Visceral samples from 60 of these fish were tested, together with ovarian fluid samples from 49 of them (Table 7). No virus was detected. The fry that hatched from eggs taken from these fish were examined in December, but also failed to yield any virus isolates.

In January, 1976, steelhead trout being spawned at Round Butte Hatchery were analyzed. Ovarian fluid and visceral samples from 60 fish were tested (Table 7). The IHN virus was isolated from several of the ovarian fluid samples and its identity confirmed. These adult fish showed no signs of disease and were apparently serving as carriers. They were considered the probable source of the virus responsible for the epizootic during April, 1975. Steelhead fry that hatched from eggs obtained from these adults suffered a severe epizootic caused by IHN virus soon after hatching and were consequently destroyed.

The only other hatchery in which IHN virus was detected during the course of this study was at Elk River located near the coast in southern Oregon. In January, 1976, 50 fall chinook salmon being spawned were examined, using both visceral and ovarian fluid samples. Infectious hematopoietic necrosis virus was isolated from all of the fluid and several of the visceral samples (Table 6). Several weeks after the eggs taken from these fish hatched, the fry suffered an epizootic caused by the virus. The adult females were free of signs and harbored the virus in the carrier state. It was again isolated from adult fall chinook at the hatchery in November, 1976.

In addition to the extensive survey of Oregon hatcheries for salmonid viruses a number of lakes and rivers throughout the state were also studied. The waters which were examined and the results obtained are shown in Table 8. The IHN virus was detected in only two cases. In August of 1971, an epizootic occurred in a population of adult rainbow trout in Nan-Scott Lake, a private lake in the Willamette River drainage (Fig. 5). A virus was isolated from the kidney tissue of diseased fish and identified as IHN. There was extensive mortality and signs were characteristic of the disease. These fish were from a private trout farm in the state of Washington and it is probable that they were carrying the virus at the time they were released in the lake. This was the only IHN epizootic observed in which adult fish were involved.

Because of the 1975 IHN epizootic at the Wizard Falls Hatchery, the possible role of the kokanee salmon population in the Metolius River as a source of the virus was considered. The river flows past the hatchery, although most of the hatchery water supply is from springs. Thirty adult kokanee were collected from the spawning grounds upstream from the hatchery and visceral samples were tested. IHN virus was isolated from these specimens (Table 8). However the possible role of these virus carrying fish in the 1975 epizootic is not clear. On the assumption that some relationship may have existed, and because of other fish disease problems such as ceratomyxosis, the Oregon Department of Fish and Wildlife quit using a series of ponds at the hatchery supplied with river water.

ISOLATIONS OF THE VIRUS OF INFECTIOUS PANCREATIC NECROSIS (IPN) IN OREGON

The virus of infectious pancreatic necrosis (IPN) was detected for the first time in Oregon salmonids in September, 1971 by J. S. McMichael. In August, the IHN epizootic among rainbow trout in Nan-Scott Lake was in progress. It was of interest to determine whether native stocks of fish inhabiting those creeks into which the lake drained might also be infected with IHN virus. A sample of 11 adult cutthroat trout were collected from these streams and tested for presence of the virus. No IHN virus could be detected, but instead these fish were found to be asymptomatic carriers of a strain of the IPN virus (Table 8).

A second isolation of IPN virus was made in October of 1971 at the Bonneville Hatchery.

Visceral samples were collected from 60 Columbia River coho salmon which were being spawned at Bonneville. There were 12 five-fish pools. All pools were inoculated after homogenization and dilution onto micro-plates cultures of both cell lines used. No changes developed on the steelhead cells (STE-137), but two of the 12 sample pools produced CPE on the chinook salmon line (CHSE-214). This was shown to be due to the presence of IPN virus, and indicated that these coho were also apparently healthy carriers of this virus (Table 4). Adult coho and fall chinook salmon were examined at this hatchery again in September and October, 1972 though no further virus isolations were made. However, specific antibody against IPN virus was detected in both species. This indicated that the fish had at some time contacted the virus, perhaps as an asymptomatic infection.

In February, 1972, two examinations were made of juveniles hatched from eggs taken at Bonneville from infected coho. On both occasions the fish were found to be infected with IPN virus, and were without specific antibody for the agent (Table 4). Despite the presence of the virus, no unusual degree of mortality attributable to it was observed among the juveniles in the hatchery. On 21 March, three weeks after the second isolation of virus, a third examination was made. No virus was recovered but antibody against the coho strain of IPN was detected. Again, no unusual mortality among juveniles in the hatchery was observed. A fourth examination in July, 1972 yielded the same results in that no virus was isolated, but specific antiviral antibody was detected.

Adult coho spawned at Bonneville in September and October, 1972 yielded no virus, even though the total number of fish sampled was increased from the usual 60 to 210. These same fish did possess antibody against IPN virus, indicating previous exposure to the agent. Fall chinook salmon spawned at the same station were likewise free of virus, but also possessed antiviral antibody (Table 4).

In October, 1972, IPN virus was isolated from adult coho salmon returning to another of the Columbia River hatcheries (Cascade). Several of these fish apparently harbored the virus, but once again were serving as asymptomatic carriers. Although offspring from this spawning were separated and repeatedly sampled, no viruses were recovered.

No other isolations of IPN virus were made during 1972, but specific antibody for this virus was detected in adult coho salmon returning to the Sandy Hatchery situated only a few miles upstream from the Columbia on the Sandy River (Table 4).

In May and June of 1973, the first hatchery epizootic of infectious pancreatic necrosis ever reported in Oregon took place. This occurred at the Fall River Hatchery of the Oregon Department of Fish and Wildlife. Juvenile eastern brook trout derived from eggs taken from East Lake were the fish chiefly affected. Mortality was high and the signs were characteristic of IPN infection. No bacterial or protozoal pathogens were found to be implicated and IPN virus was isolated and identified from moribund animals. Fewer deaths occurred in another population of brook trout hatched from eggs taken at Elk and Big Lava Lakes, and juvenile and yearling rainbow trout at the hatchery were asymptomatic. However the virus was isolated from these groups of fish as well. Specific antiviral antibody was also found in the blood of fish from all of these groups. At the time of the epizootic the virus was isolated from brook trout taken both below and above the hatchery, although it was not detected in either rainbow or brown trout taken from these sites at that time.

As a result of this epizootic, certain salmonid populations in central Oregon were examined for viral agents in the summer of 1973. This survey detected the presence of IPN virus in eastern brook trout in Elk Lake, Big Lava Lake and Fish Lake. Antibody for this virus was also detected in these fish, as well as in fish of the same species at the Wizard Falls Hatchery.

In September, 1973, Fall River and its tributaries were treated with rotenone to eliminate the fish populations inhabiting these waters. Hatchery facilities were thoroughly disinfected, and the hatchery was not used for nine months.

No IPN virus has been detected in the brook trout population of East Lake, though numerous samples have been tested. In view of the presence of IPN virus in both Elk and Big Lava lakes, it was concluded that eggs taken from the latter two sources carried the virus to the hatchery and ultimately caused the eipzootic.

IPN virus was isolated three other times during 1973. In September, 30 adult spring chinook salmon were examined and tested at the Round Butte Hatchery. They were found

to be asymptomatic carriers of the virus (Table 7). In December, 60 adult rainbow trout at the Roaring River Hatchery were sampled and the ovarian fluids divided into 12 five-fish pools. One of the 12 pools yielded an isolate of IPN virus. Some of the fish were asymptomatic carriers. No outbreak of disease is known to have occurred among fry from their eggs held at the hatchery. The virus was also isolated from a sample of 60 juvenile spring chinook salmon being held at the Oregon Department of Fish and Wildlife Research Laboratory in Corvallis. They were not showing signs of disease, and were apparently asymptomatic carriers. They had been received as eyed eggs from Cole Rivers Hatchery. Accordingly, about 150 spring chinook salmon juveniles from the same source were obtained from the hatchery and analyzed for the presence of a fish virus. None could be detected.

In January of 1974, the second IPN epizootic in an Oregon hatchery occurred. Eggs taken from brook trout in East Lake during the fall of 1973 were transported to Wizard Falls Hatchery. In January the progeny from these eggs began to die in large numbers with signs suggesting a virus infection. IPN virus was isolated from several samples of moribund fish and its identity confirmed by reaction with specific antibody (Table 7). Almost 700,000 juvenile brook trout were either killed by the disease or had to be destroyed. The ponds that held the infected fish were disinfected and allowed to dry, and the hatchery building sanitized. A group of these fish that had been removed from the hatchery as eggs and placed in the laboratory for experimental use were tested, but no virus was isolated.

While the Wizard Falls IPN epizootic was in progress, a sample of 50 juvenile brown trout from Fort Klamath Hatchery was collected and tested for the possible presence of a virus. These fish were not experiencing abnormal mortality despite the fact that they were found to be harboring IPN virus. The source of the eggs from which the brown trout hatched was also East Lake. The fact that both these fish and the brook trout in the IPN epizootic were derived from East Lake fish may cast doubt on the suitability of this lake as an egg source. This was despite the fact that many adequate samples of East Lake brook and brown trout had been tested during 1973 and no fish virus had ever been detected (Table 8).

Thus, the available evidence is conflicting. Although the infected brook trout at the Wizard Falls Hatchery were originally derived from East Lake fish, the extensive examination and virological analysis performed on East Lake salmonid populations should have revealed the presence of IPN if they harbored the virus. It must be concluded that the source of IPN virus in the 1974 epizootic at Wizard Falls remains unknown.

During the summer of 1974, lakes of the Cascade mountains in central Oregon were surveyed for virus. The results of this survey are shown in Table 8. Figure 5 shows the location of the lakes. The presence of IPN virus in the brook trout of Elk and Big Lava Lakes has already been mentioned. Only three other lakes of the considerable number examined yielded any virus isolates. These were Cache Lake, Sparks Lake and Fish Lake. In each case the virus isolated was IPN and the salmonid species was brook trout.

In March, 1975, another epizootic occurred at the Wizard Falls Hatchery. Both IPN and IHN viruses were isolated from the affected fish. Details of this epizootic were discussed in the preceeding section concerning isolations of IHN virus. In an effort to reveal the source of the IPN virus in the 1975 Round Butte Hatchery epizootic, 13 isolates of the virus from Oregon waters and hatcheries were compared antigenically by means of cross neutralization measurements with specific antisera against each isolate. The results were then compared statistically. A great deal of minor antigenic diversity was found between strains, but the 1975 Wizard Falls strains and the strain from Roaring River Hatchery were closely related. This suggested that the Roaring River strain was the probable source of the virus at Wizard Falls in 1975, despite conflicting evidence to the contrary. The analysis also suggested that the IPN strain in the 1974 epizootic at Wizard Falls was not the same and probably derived from a different source.

All infected stocks of fish at Wizard Falls Hatchery in 1975 were destroyed and the hatchery was disinfected. No viral epizootic occurred at this hatchery in 1976 indicating that the disinfection process had been successful. Furthermore, juvenile populations of kokanee salmon, brook and brown trout, and Atlantic salmon at the hatchery have been examined for the possible presence of fish viruses with negative results.

DETECTION OF VIRAL ERYTHROCYTIC NECROSIS (VEN) IN OREGON COASTAL WATERS

The presumed virus of viral erythrocytic necrosis (formerly piscine erythrocytic necrosis, or PEN) has not yet been isolated in cell cultures. Its presence has only been detected by observation of the typical pathological changes in erythrocytes, and by the presence of the hexagonal or pentagonal particles seen by electron microscopy in the cytoplasm of infected cells.

The virus was first identified in Oregon by Marie Philippon-Fried in diseased adult chum salmon *(Oncorhynchus keta)* returning to the OSU Experimental Hatchery at Netarts Bay in 1976 (Fig. 5). It was also observed in this species in 1978 and 1979. These observations were confirmed by Olson (1977). Philippon-Fried also found it in 1976 in chum salmon from the Kilchis River, which flows into Tillamook Bay, Oregon and in Pacific herring *(Clupea harengus pallasi)* from Yaquina Bay, Oregon (Fig. 5). The morphology of the presumed virus particles from chum salmon is shown in Figure 1c.

The most complete report of VEN infection in salmonids on the Pacific coast is that of Evelyn and Traxel (1978), who encountered it frequently in chum and pink salmon. They added valuable information concerning transmission of the virus, and their findings have been summarized in the section of this report on "Characteristics of the Virus". MacMillan and Mulcahy (1979) found the disease in chum salmon and Pacific herring from Puget Sound, Washington, and provided additional information on experimental transmission to other salmonid species.

recommendations for control

1. Because of the high incidence of both IPN and IHN viruses from fish in the Deschutes River watershed, any movements of salmonids from this area (except Oak Springs and Wizard Falls Hatcheries) to another should be strongly discouraged.

2. Only testing of ovarian fluids can be considered adequate for detecting IHN virus, especially in asymptomatic carriers. Testing of homogenates of entire alevin or juvenile fish, or of visceral samples, cannot be considered satisfactory evidence for the absence of IHN virus.

3. The discovery of IHN virus at Elk River Hatchery, the only virus isolated from fish in a coastal watershed indicates that the distribution of these agents is subject to change. Only continued examination of broodstocks (hatchery and feral) can assure an adequate estimation of the status of virus in fish in any watershed or rearing facility.

4. Before a salmon run can be considered free of viruses, consecutive examination over a three to five-year period (depending on species) should be performed on spawning adults.

5. Between 1973 and 1978, IPN virus was isolated from fish in at least one hatchery each year and several epizootics occurred. By destruction of infected stocks, extensive disinfection procedures and identification of virus-free broodstocks epizootics have been controlled and recurrences prevented. Aggressive management is warranted to prevent the reintroduction of this virus in Oregon hatcheries and to control IHN virus in the salmon hatcheries where it remains a problem.

6. Adult carrier trout and salmon are asymptomatic and appear healthy. However, they should be considered unsatisfactory for routine stocking and release because of the danger of extending the distribution of the fish viruses. Stocking of virus-infected fish should be done only as a last resort and then only when necessary to prevent irreparable harm to a population. These fish might best be released in areas already known to be contaminated by the virus they are carrying. Stocking of virus-infected trout should be discouraged and the release of virus-infected salmon should be done with the realization that many of these fish will return as virus-carrier adults and therefore use of the fish as an egg source will perpetuate the disease problem.

7. Efforts should be made to examine more populations of salmonids for fish viruses in eastern Oregon.

8. Before any new hatchery or other fish production facility is built a survey of the feral fish in the area should be made for pathogens. No new facility should be built in any of the watersheds known to be contaminated with fish viruses unless that facility has a virus-free water supply.

9. Any feral fish, including salmon or steelhead returning to a hatchery in the Columbia, Snake, Elk, Klamath, Deschutes or Willamette watersheds, should be considered as potential IPN or IHN virus-carriers because these viruses have been previously isolated in fish from these watersheds.

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appendix

The following abbreviations are used in Tables 4-8, which appear in the Appendix.

A. Abbreviations for fish species.

Br	brown trout	ChS	spring chinook salmon
BT	brook trout	Co	coho salmon
Ct	cutthroat trout	ST	steelhead trout
Ct Lah	Lahontan cutthroat trout	StS	summer steelhead trout
K	kokanee salmon	StW	winter steelhead trout
Rb	rainbow trout	SS	sockeye salmon
AS	Atlantic salmon	ChuS	chum salmon
RB-Red	red banded rainbow trout	PaH	Pacific herring
ChF	fall chinook salmon		

B. Abbreviations for fish tissues collected for virus examinations.

V	visceral specimens, including kidney, spleen and sometimes liver
OF	ovarian fluid
S	sperm
WF	whole fish
K	kidney

C. Abbreviations for stage of development.

Al	Alevin
Juv	Juvenile
Ad	Adult

D. Other abbreviations.

NR	Not recorded
-N-	None

Table 4. Viral examinations of salmonid fish from Oregon hatcheries in the Columbia River system.

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Klaskanine	11/72	60	Co	Ad	12 V 12 OF	None
	3/73	50	Co	Juv	5 WF	None
	11/73	60	Co	Ad	12 V 12 OF	None
	1/74	60	Co	Al	6 WF	None
	11/74	60	Co	Ad	12 V 12 OF	None
	3/77	60	St	Ad	12 V 12 OF	None
	11/77	60	Co	Ad	12 V 12 OF	None
	12/77	10	ChF	Ad	1 S	None
	11/78	60	Co	Ad	12 V 12 OF	None
Big Creek	2/72	300	ChF	Juv	30 WF	None
	9/72	60	ChF	Ad	12 V 12 OF	None
	11/72	60	Co	Ad	12 V 12 OF	None
	12/72	50	ChF	Al	5 WF	None
	1/73	60	St	Ad	12 V 12 OF	None
	4/73	50	St	Juv	5 WF	None
	10/73	60	ChF	Ad	12 V 12 OF	None
	11/73	60	Co	Ad	12 V 12 OF	None
	1/74	60	ChF	Juv	6 WF	None

Table 4 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Big Creek	1/74	60	Co	Al	6 WF	None
	11/76	60	Co	Ad	12 V 12 OF	None
	1/77	32	St	Ad	8 V 8 OF	None
	9/77	60	ChF	Ad	12 V 12 OF	None
	11/77	60	Co	Ad	12 V 12 OF	None
	11/77	60	Co	Ad	12 V 12 OF	None
	1/78	60	St	Ad	12 V 12 OF	None
	5/78	20	St[1]	Juv	2 WF	None
	7/78	60	Co	Juv	12 V	None
	7/78	60	Co[2]	Juv	12 V	None
	9/78	60	ChF	Ad	12 V 12 OF	None
	11/78	60	Co	Ad	12 V 12 OF	None
Prescott[3]	2/72	10	St	Ad	2 V	None
	4/75	60	St	Ad	12 V	None
	5/76	60	St	Ad	12 V	None

Table 4 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Cascade	2/72	300	Co[4]	Juv	30 WF	IPN
	10/72	60	Co	Ad	12 V 12 OF	IPN
	12/72	50	ChF	Al	5 WF	None
	1/73	150	Co	Al	15 WF	None
	5/73	50	Co	Juv	10 V	None
	9/73	60	ChF	Ad	12 V 12 OF	None
	10/73	60	Co	Ad	12 V 12 OF	None
	2/74	60	ChF	Juv	6 WF	None
	2/74	60	Co	Juv	6 WF	None
	9/77	60	ChF	Ad	12 V 12 OF	None
	9/78	60	ChF	Ad	12 V 12 OF	None
	10/78	60	Co	Ad	12 V 12 OF	None
Sandy	11/72	60	Co	Ad	12 V 12 OF	None
	3/73	50	Co	Juv	5 WF	None
	10/73	60	Co	Ad	12 V 12 OF	None
	11/74	60	Co	Ad	12 V 12 OF	None
	10/75	60	Co	Ad	12 V 12 OF	None
	1/76	60	ChF	Juv	6 WF	None

Table 4 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Sandy	10/76	120	Co	Ad	24 V 24 OF	None
	11/77	60	Co	Ad	12 V 12 OF	None
	8/78	60	Co	Juv	12 V	None
	11/78	60	Co	Ad	12 V 12 OF	None
Bonneville	9/71	60	ChF	Ad	12 V 12 OF	None
	10/71	60	Co	Ad	12 V 12 OF	IPN
	2/72	300	Co	Al	30 WF	IPN
	2/72	300	Co	Al	30 WF	IPN
	3/72	300	Co	Juv	30 WF	None[5]
	3/72	60	Co	Juv	12 V	None
	3/72	60	ChF	Juv	12 V	None
	7/72	60	Co	Juv	12 V	None[5]
	9/72	60	ChF	Ad	12 V 12 OF	None[5]
	9/72	150	ChF	Ad	30 V 30 OF	None[5]
	10/72	210	Co	Ad	42 V 42 OF	None[5]
	12/72	50	ChF	Juv	5 WF	None
	2/73	50	Co	Juv	5 WF	None
	9/73	60	ChF	Ad	12 V 12 OF	None

Table 4 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Bonneville	10/73	60	Co	Ad	12 V 12 OF	None
	2/74	60	ChF	Juv	6 WF	None
	2/74	60	Co	Juv	6 WF	None
	9/74	60	ChF	Ad	12 V 12 OF	None
	11/74	60	Co	Ad	12 V 12 OF	None
	10/76	60	Co	Ad	12 OF	None
	9/77	60	ChF	Ad	12 V 12 OF	None
	11/77	60	Co	Ad	12 V 12 OF	None
	7/78	60	Co	Juv	12 V	None
	7/78	60	Co[6]	Juv	12 V	None
	8/78	60	ChF	Juv	12 V	None
	8/78	60	ChF[7]	Juv	12 V	None
	8/78	60	ChF[8]	Juv	12 V	None
	9/78	60	ChF	Ad	12 V 12 OF	None
	10/78	60	Co	Ad	12 V 12 OF	None
Oxbow	2/72	300	ChF	Juv	30 WF	None
	3/73	60	ChF	Juv	12 V	None
	9/77	9	ChS	Ad	2 V 2 OF	None

Table 4 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Oxbow	9/77	8	ChS	Ad	2 V 2 OF	None
	9/77	5	ChS	Ad	1 V 1 OF	None
	9/77	8	ChS	Ad	2 V 2 OF	None

1. Fish reared at Gnat Creek Hatchery.
2. Fish were from Cowlitz River stock, Washington State.
3. Prescott is an experimental fish holding facility, not a hatchery.
4. Fish were from Bonneville Hatchery stock.
5. Antibody for IPN virus was detected in these specimens. Examination of serum for antibody was not done routinely.
6. Fish were from 1976 brood, Bonneville Hatchery, being held at the Oregon State University Fish Disease Laboratory, Corvallis, Oregon.
7. Fish collected from the Ladder at Bonneville Dam.
8. Fish were from Cowlitz River Hatchery stock.

Table 5. Viral examinations of salmonid fish from Oregon hatcheries in the Willamette River drainage.

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Roaring River	7/71	4	Rb	Ad Br	NR	None
	7/71	8	Ct	Ad Br	NR	None
	12/71	57	Rb	Ad Br	12 V	None
	1/72	5	Ct	Ad Br	NR	None
	2/72	10	St	Ad	2 V	None
	12/73	60	Rb	Ad Br	12 OF	IPN[5]
	1/74	90	Rb	Ad Br	18 OF	None
	2/74	60	Rb	Al	6 WF	None
	2/74	45	Rb	Ad Br	9 OF	None
	2/74	60	Rb	Ad Br	12 V	None
	2/74	60	St	Ad	12 V	None
	4/74	30	Rb[1]	Juv	6 V	None
	12/74	60	Rb	Ad Br	12 OF	None
	3/75	700	Rb	Juv	8 WF	None
	3/75	35	Rb	Ad Br	7 V	None
	8/75	13	Rb	Ad Br	13 V	None
	2/76	85	Rb	Ad Br	17 V	None

Table 5 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
	2/76	80	Rb[2]	Juv	8 WF	None
	11/76	60	Rb	Ad Br	12 OF	None
	3/77	45	St	Ad Br	9 V 4 OF	None
	12/77	60	St	Ad	12 OF	None
	3/78	24	StS[3]	Ad	5 V 5 OF	None
	4/78	24	StS[3]	Ad	4 V 4 OF	None
	11/78	48	Rb	Ad Br	9 OF	None
	12/78	35	Rb	Ad Br	7 OF	None
Marion Forks	9/72	60	ChS	Ad	12 V 12 OF	None
	9/72	5	ChS[4]	Ad	1 V	None
	2/73	50	ChS	Juv	5 WF	None
	9/73	60	ChS	Ad	12 V 12 OF	None
	1/74	60	ChS	Juv	6 WF	None
	5/74	40	St	Ad	8 V	None
Minto Holding Ponds	9/71	60	ChS	Ad	12 V 12 OF	None
	5/72	38	St	Ad	8 V 8 OF	None
	4/77	60	St	Ad	12 V 12 OF	None

Table 5 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Ore. Dept. Fish and Wildlife Res. Lab. Corvallis	12/73	60	ChS	Juv	6 WF	IPN
	1/74	25	Co	Ad	6 V	None
	1/74	13	Ct	Juv	6 V	None
	1/74	50	Co	Juv	3 WF	None
	1/74	50	ChS	Juv	4 WF	None
	1/74	75	ChS	Juv	5 WF	IPN
	1/74	40	ChF	Juv	4 WF	None
	1/74	16	Ct	Juv	4 V	None
	1/74	45	Co	Juv	3 WF	None
	1/74	30	Co	Ad	6 V	None
	2/74	35	ChS	Juv	3 WF	IPN
	2/74	35	ChF	Juv	3 WF	None
South Santiam	3/74	55	St	Ad	11 V 11 OF	None
	1/75	41	St	Ad	8 OF	None
	1/76	30	St	Ad	6 V 6 OF	None
	1/77	21	St	Ad	4 V 4 OF	None
	2/77	30	St	Ad	6 V 6 OF	None
	9/77	60	ChS	Ad	12 V 12 OF	None
	10/77	10	ChS	Juv	2 V	None

Table 5 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled		Virus Isolated
South Santiam	1/78	60	St	Ad	12 V	12 OF	None
	9/78	60	ChS	Ad	12 V	12 OF	None
	9/78	10	St	Juv	2 V		None
	1/79	60	St	Ad	12 V	12 OF	None
Leaburg	1/74	40	Ct	Ad Br		8 OF	None
	6/76	10	Rb	Juv	2 V		None
	1/77	60	Ct	Ad Br		12 OF	None
	1/78	60	Ct	Ad Br	4 V	12 OF	None
McKenzie	9/78	60	ChS	Ad	12 V	12 OF	None
Dexter Holding Ponds	9/71	60	ChS	Ad	12 V		None
	9/72	60	ChS	Ad	12 V	12 OF	None
	9/78	60	ChS	Ad	12 V	9 OF	None
Oakridge	9/58	72	SS	Juv		50 K	IHN
	6/59	44	SS	Juv		30 K	IHN

Table 5 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Willamette	1/72	60	Rb	Ad Br	12 V	None
	1/74	60	Rb	Ad Br	12 OF	None
	5/75	11	Rb	Ad Br	2 V	None
	12/75	26	Rb	Ad Br	5 OF	None
	1/76	26	Rb	Ad Br	5 OF	None
	1/77	6	Rb	Ad Br	1 V	None
	1/77	60	Rb	Ad Br	12 OF	None
	10/77	3	Rb	Ad Br	1 V	None
	1/78	60	Rb	Ad Br	12 OF	None
	1/78	60	Rb	Ad Br	12 OF	None
	12/78	60	Rb	Ad Br	12 OF	None

1. Fish were held at the Western Fish Toxicology Laboratory, Corvallis, Ore.
2. Fish were reared at Oak Springs Trout Hatchery.
3. Fish were from Siletz River Stock.
4. These fish were landlocked.
5. Isolation of IPN virus could not be confirmed.

Table 6. Viral examinations of salmonid fish from Oregon Hatcheries on coastal rivers.

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Nehalem	11/72	60	Co	Ad	12 V 12 OF	None
	3/73	50	Co	Juv	5 WF	None
	11/77	60	Co	Ad	12 V 12 OF	None
Trask	11/73	30	Co	Ad	12 V 12 OF	None
	4/74	30	Co[1]	Juv	6 V	None
	9/76	60	ChS	Ad	12 V 12 OF	None
	10/76	60	Co	Ad	12 V 12 OF	None
	9/77	60	ChS	Ad	12 V 12 OF	None
	11/77	60	ChF	Ad	12 V 12 OF	None
	12/77	20	ChF	Ad	5 V 4 OF	None
	9/78	60	ChS	Ad	12 V 12 OF	None
	11/78	48	ChF	Ad	9 V 9 OF	None
	12/78	25	ChF	Ad	5 V 5 OF	None
Siletz	11/73	60	Co	Ad	12 V 12 OF	None
	3/74	60	Co	Juv	6 WF	None
	3/74	30	St[3]	Ad	6 V	None
	4/75	28	St[3]	Ad	5 V	None
	11/75	45	Co	Ad	9 V	None
	3/76	60	Co[2]	Juv	6 WF	None
	4/76	60	St[3]	Ad	12 V	None

Table 6 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Siletz	5/76	60	Co[2]	Juv	12 V	None
	11/76	60	Co	Ad	12 V 12 OF	None
	3/77	25	St[3]	Ad	5 V 5 OF	None
	11/77	60	Co	Ad	12 V 10 OF	None
	3/78	24	St[3]	Ad	5 V 5 OF	None
	11/78	59	Co	Ad	12 V 12 OF	None
Fall Creek	12/71	60	Co	Ad	12 V	None
	11/72	60	Co	Ad	12 V 12 OF	None
	3/73	50	Co	Juv	5 WF	None
	11/73	60	Co	Ad	12 V 12 OF	None
	3/74	60	Co	Juv	6 WF	None
	11/74	60	Co	Ad	12 V 12 OF	None
	10/75	60	Co	Ad	12 V	None
	10/76	25	ChF	Ad	5 V 3 OF	None
	11/76	60	Co	Ad	12 V 12 OF	None
	11/76	6	ChF	Ad	1 V 1 OF	None
	11/76	4	ChF	Ad	1 V 1 OF	None

Table 6 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled		Virus Isolated
Fall Creek	11/76	11	ChF	Ad	2 V	1 OF	None
	12/76	3	ChF	Ad	1 V	1 OF	None
	10/77	46	ChF	Ad	9 V		None
	11/77	10	ChF	Ad	2 V	2 OF	None
	11/77	30	Co	Ad	6 V	6 OF	None
	11/77	30	Co	Ad	6 V	6 OF	None
	11/77	20	ChF	Ad		4 OF	None
	11/77	30	ChF	Ad		6 OF	None
	11/77	14	ChF	Ad	2 V	1 OF	None
	11/77	10	ChF	Ad		2 OF	None
	11/77	60	Co	Ad	12 V		None
	12/77	2	Co	Ad		1 OF	None
	11/78	60	Co	Ad	12 V		None
	12/78	23	ChF	Ad	1 V	4 OF	None
Alsea	1/72	55	Ct	Ad Br	11 V		None
	1/74	60	Ct	Ad Br	12 V		None
	5/74	20	StW	Juv	2 WF		None
	2/76	76	St	Ad		15 OF	None
	3/77	60	St	Ad	12 V	12 OF	None
	1/78	60	Ct	Ad Br		12 OF	None
	1/78	60	StW	Ad		12 OF	None

Table 6 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Bandon	1/75	45	Ct	Ad Br	9 OF	None
	5/77	3	Ct	Juv	1 V	None
	6/77	30	Ct	Juv	6 V	None
	6/77	60	StW[4]	Juv	12 V	None
	6/77	60	StS[5]	Juv	12 V	None
	2/78	60	Ct	Ad Br	12 OF	None
Rock Creek	3/76	76	St	Ad	15 OF	None
Elk River	12/72	60	ChF	Ad	12 V 12 OF	None
	1/76	50	ChF	Ad	12 V 12 OF	IHN
	3/76	60	ChF	Al	12 WF	IHN
	4/76	280	ChF	Juv	28 WF	IHN
	5/76	60	ChF	Juv	6 WF	IHN
	5/76	30	ChF[6]	Juv	3 WF	None
	5/76	60	ChF	Juv	6 WF	None
	6/76	60	ChF[6]	Juv	6 WF	None
	9/76	20	ChF	Juv	4 V	None

Table 6 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Elk River	11/76	60	ChF	Ad	12 V 12 S	IHN
	11/76	16	ChF[6]	Ad	V S and OF	None
	12/76	19	ChF[6]	Ad	4 V 4 OF	None
	1/77	36	ChF[6]	Ad	6 V 5 OF	None
	2/77	10	StW[6]	Ad	2 OF	None
	3/77	12	StW[6]	Ad	1 V 3 OF	None
	3/77	1	ChF	Ad	1 V 1 OF	None
	11/77	73	ChF	Ad	7 V 15 OF	None
	12/77	114	ChF	Ad	22 V 22 S or OF	None
	1/78	23	ChF	Ad	4 V 4 S or OF	None
	5/78	10	ChF	Juv	1 WF	IHN
	5/78	20	ChF	Juv	2 WF	None
	12/78	74	ChF	Ad	14 OF	IHN
	12/78	134	ChF	Ad	30 OF	IHN
Chetco[7] River	11/76	26	ChF	Ad	26 V 1 OF	None
	11/77	13	ChF	Ad	6 V 4 OF	None
	12/77	127	ChF	Ad	7 V 26 OF	IHN
	2/78	16	ChF	Ad	2 V 2 OF	None

Table 6 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Cole Rivers	1/74	150	ChS	Al	15 WF	None
	6/74	30	St	Juv	3 WF	None
	3/75	60	Rb	Juv	6 WF	None
	2/77	42	Rb	Ad Br	8 OF	None
	3/77	30	St	Ad	6 V	None
	3/77	55	St	Ad	11 OF	None
	9/77	20	ChS	Ad	4 V 4 OF	None
	11/77	30	Co	Ad	6 V 6 OF	None
	12/77	60	Rb	Ad Br	12 OF	None
	9/78	60	ChS	Ad	12 V 12 OF	None
Butte Falls	3/75	100	Rb	Juv	10 WF	None

1. Fish were reared at the Western Fish Toxicology Laboratory, Corvallis, Ore.
2. Fish were reared at Oregon Aquafoods' Wright Creek Hatchery.
3. Fish were held at the Roaring River Trout Hatchery.
4. Fish were from Alsea Hatchery stock.
5. Fish were from Umpqua River stock.
6. Fish were from Chetco River stock.
7. Adult fall chinook salmon and steelhead trout were seined in the river or trapped at a facility on Jack Creek, a tributary of the Chetco River. Egg incubation and rearing to smolts were conducted at Elk River Hatchery.

Table 7. Viral examinations of salmonid fish from central and eastern Oregon hatcheries.

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Oak Springs	1/72	58	Rb	Ad Br	12 V 12 OF	None
	4/74	60	Rb	Ad Br	12 V	None
	4/74	15	Rb	Juv	3 WF	None
	4/75	60	Rb	Juv	12 WF	None
	4/75	25	St	Juv	5 WF	None
	5/75	60	St	Juv	12 V	None
	2/76	80	Rb[1]	Juv	8 WF	None
	11/76	60	Rb	Ad Br	12 OF	None
	9/77	60	St	Juv	12 V	None
	11/77	60	Rb	Ad Br	4 V 12 OF	None
	10/78	60	Rb	Ad Br	12 V 12 OF	None
Round Butte	11/71	19	ChS	Ad	4 V 4 OF	None
	8/73	60	ChS	Ad	12 OF	IHN[2]
	8/73	25	ChS	Juv	5 V	None
	9/73	30	ChS	Ad	6 V	IPN
	10/73	22	K	Ad	4 V	None
	10/73	27	St	Ad	5 V	None

Table 7 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Round Butte	10/73	50	St	Juv	5 V	None
	12/73	150	ChS	Juv	15 WF	None
	1/74	150	ChS	Juv	15 WF	None
	5/74	50	St	Juv	7 WF	None
	10/74	60	St	Juv	12 V	None
	10/74	20	ChS	Al	4 WF	None
	1/75	30	St	Ad	6 V	None
	4/75	60	St	Juv	12 WF	IHN
	4/75	60	St	Juv	12 WF	IHN
	5/75	60	St	Juv	12 V	None
	5/75	135	St	Juv	9 WF	IHN
	5/75	45	ChS	Juv	3 V	None
	5/75	60	St	Ad	12 V	None
	5/75	90	ChS	Juv	18 V	None
	6/75	120	St	Juv	4 WF	None
	9/75	16	ChS	Ad	3 V	None
	9/75	6	ChS	Ad	1 OF	None
	9/75	12	ChS	Ad	3 V	None
	9/75	16	ChS	Ad	3 V 4 OF	None

Table 7 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Round Butte	12/75	20	ChS	Juv	4 WF	None
	1/76	60	St	Ad	12 V 12 OF	IHN
	5/76	60	St	Juv	6 WF	IHN
	3/77	12	St	Ad	3 V 3 OF	IHN
	3/77	12	St	Ad	3 V 3 S	IHN
	3/77	13	St	Ad	V OF	IHN
	3/77	13	St	Ad	V	IHN
	3/77	12	St	Ad	V OF	IHN
	3/77	12	St	Ad	V	None
	5/77	240	St	Juv	24 WF	None
	6/77	420	St	Juv	42 WF	IPN
	6/77	360	St	Juv	36 WF	None
	3/78	60	St	Ad	12 OF	IHN
	3/78	60	St	Ad	12 V	None
	5/78	10	St	A1	1 WF	IHN
	9/78	10	St	Juv	2 V	None
Wizard Falls	7/73	24	BT	Ad	6 V	None
	1/74	200	BT	Juv	12 WF	IPN
	2/74	45	BT	Juv	3 WF	IPN
	3/74	150	RB	Juv	14 WF	None
	6/74	100	St[3]	Juv	3 WF	None
	6/74	30	AS	Ad	6 V	None
	3/75	40	K	Juv	3 WF	IHN

Table 7 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Wizard Falls	3/75	36	Rb	Juv	3 WF	IPN/IHN
	3/75	80	Rb	Juv	6 WF	IPN/IHN
	3/75	100	K	Juv	6 WF	IHN
	3/75	75	K	Juv	6 WF	IHN
	3/75	60	Rb	Juv	6 WF	IPN/IHN
	4/75	30	AS	Ad	6 V	None
	4/75	30	St	Ad	6 V	None
	4/75	100	Rb	Ad	12 V	None
	4/75	60	AS	Juv	6 WF	None
	4/75	40	Rb	Juv	4 WF	IPN/IHN
	4/75	100	Rb	Juv	10 WF	IHN
	4/75	30	BT	Ad	6 V	None
	6/75	60	Rb	Juv	6 WF	None
	6/75	30	AS	Juv	6 V	None
	10/75	10	Rb	Ad	12 V	None
	1/76	30	K	Juv	6 WF	None
	1/76	15	BT	Juv	3 WF	None
	1/76	15	Br	Juv	3 WF	None
	1/76	15	AS	Juv	3 WF	None

Table 7 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Wizard Falls	5/76	60	BT	Juv	6 WF	None
	11/77	60	BT	Ad Br	12 OF	None
	3/78	30	K	Juv	3 WF	None
	10/78	9	AS	Ad Br	2 OF	None
	12/78	10	Rb	Juv	1 WF	None
Fall River	6/73	50	BT[4]	Juv	5 WF	IPN
	6/73	20	BT[4]	Juv	2 WF	IPN
	6/73	20	BT[5]	Juv	2 WF	IPN
	6/73	20	Rb	Juv	2 WF	IPN
	6/73	20	BT[4]	Juv	2 WF	IPN
	6/73	20	BT[5]	Juv	2 WF	IPN
	6/73	20	Rb	Juv	2 WF	IPN
	6/73	60	Rb	Juv	12 V	IPN
	6/73	4	BT[6]	Ad	1 V	IPN
	6/73	20	BT[7]	Juv	4 V	None
	6/73	20	BT[7]	Juv	4 V	None
	6/73	20	BT[7]	Juv	4 V	None
	9/73	5	Rb[7]	Ad	1 V	None
	9/73	25	BT[7]	Ad	5 V	IPN

Table 7 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Fall River	9/73	3	BT[8]	Ad	1 V	None
	9/73	3	Rb[8]	Ad	1 V	None
	9/73	3	Br[8]	Ad	1 V	None
	9/73	10	BT[6]	Ad	2 V	IPN
	9/73	5	Br[6]	Ad	1 V	None
	3/74	200	BT	Al	20 WF	None
	3/74	200	BT	Al	20 WF	None
	6/74	34	Rb	Ad Br	7 V	None
	6/74	30	BT	Juv	6 WF	None
	1/75	400	BT	Al	8 WF	None
	2/75	700	BT	Al	21 WF	None
	4/75	400	BT	Juv	8 WF	None
	5/75	120	BT	Juv	12 WF	None
	2/76	200	BT	Al	4 WF	None
	2/76	40	BT[9]	Al	4 WF	None
Fort Klamath	6/73	20	Br[10]	Ad	5 V	None
	6/73	20	Br[11]	Ad	5 V	None
	1/74	50	Br	Juv	3 WF	IPN
	2/74	60	Rb	Ad	12 OF	None
	2/74	30	Rb	Juv	2 WF	None

Table 7 (continued)

Hatchery or Location	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Fort Klamath	4/74	60	Br	Ad	13 V	None
	8/74	16	Rb	Juv	4 WF	None
	1/75	100	Rb	Juv	16 WF	None
	3/75	40	Rb	Juv	3 WF	None
	4/75	60	Br	Juv	12 WF	None
	8/77	20	Ct Lah	Juv	2 WF	None
Wallowa	6/76	58	St	Ad	12 V 12 OF	None
	4/77	16	StW	Ad	3 V 3 OF	None
	5/77	12	StW	Ad	2 V 2 OF	None
	5/77	8	StW	Ad	2 V 2 OF	None
	6/77	NR	St	Ad	1 V 1 OF	None
	4/78	40	StW	Ad	10 V 10 OF	None

1. Fish from Roaring River Hatchery.
2. Virus isolated and identified by S. Leek, Bureau of Sport Fisheries and Wildlife, and confirmed at the Western Fish Disease Laboratory, Seattle, Wash. by D. F. Amend.
3. Fish from Umatilla River.
4. From East Lake.
5. From Lava Lake.
6. Fish collected from Fall River downstream from the hatchery.
7. Fish collected from Fall River upstream from the hatchery.
8. Fish collected from Fall River adjacent to the hatchery.

Table 7 (continued)

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9. Fish were from Little Cultus Lake.

10. Fish were from Suttle Lake.

11. Fish were from East Lake.

Table 8. Viral examinations of salmonid fish from Oregon lakes and rivers.

A. Waters located in the coastal zone and in the Willamette Valley.

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Nan Scott Lake	8/71	15	Rb	Ad	3 V	IHN
Outlet Cr. Nan Scott L.	9/71	11	Ct	Ad	3 V	IPN
Yaquina R.	11/78	25	ChF	Ad	10 V 7 OF	None
	12/78	17	ChF	Ad	6 V 2 OF	None
Green Peter Res.	10/77	14	SS & K	Ad	5 V 3 OF	None
Alsea R.	12/78	22	ChF	Ad	5 V 5 OF	None
Siuslaw R.	11/78	38	ChF	Ad	12 V 9 OF	None
	12/78	12	ChF	Ad	3 V 3 OF	None
Umpqua R.	5/78	60	Br & Rb	Ad	12 V	None
	10/78	16	ChS	Ad	4 V 4 OF	None
Smith R.[1]	11/77	30	Co	Ad	6 V 6 OF	None

Table 8 (continued)

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Soda Springs[1] Pond	10/78	16	ChS	Ad	4 V 4 OF	None
Coos R.	11/76	19	ChF & Co	Ad	19V 8S 6 OF	None
	11/78	42	ChF	Ad	10 V 4 OF	None
	12/78	6	ChF	Ad	3 V 1 OF	None
Tioga Cr.[2]	12/77	12	ChF	Ad	2V 1S	None
Williams R.[2]	11/77	22	ChF & Co	Ad	4V 4S or OF	None
Coquille R.	11/77	12	ChF	Ad	3 V	None
Steel Cr.[3]	12/77	8	ChF	Ad	2V 1S 1 OF	None
Bald Mt. Cr.[4]	5/76	60	ChF & Rb	Juv	6 WF	None
	5/76	20	Rb	Ad	4 V	None

Table 8 (continued)

B. Waters located in central and eastern Oregon.

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Umatilla R.	5/74	14	St	Ad	3 V	None
Duck L.	9/75	27	BT	Ad	6 V	None
	9/75	22	Rb	Ad	4 V	None
Metolius R. (upper)	10/75	30	K	Ad	6 V	IHN
	9/76	20	K	Ad	4 V	IHN
	9/77	NR[6]	K	Ad	S	IHN
	9/78	20	K	Ad	V S OF	IHN
	10/78	8	K	Ad	V	IHN
Suttle L.	10/71	60	K	Ad	12 V	None
	6/73	20	Br[5]	Juv	2 WF	None
	9/75	68	K	Ad	14 V	None
	9/77	10	K	Ad	2 V	None
	8/78	10	K	Ad	2 V	None
	10/78	60	K	Ad	12 V	None
	11/78	9	Br	Ad Br	2 OF	None
Hand L.	7/74	2	Rb	Ad	1 V	None

Table 8 (continued)

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Cache L.	7/74	3	BT	Ad	1 V	IPN
	7/74	1	Ct	Ad	1 V	None
Link L.	7/74	1	Rb	Ad	1 V	None
Meadow L.	7/74	3	BT	Ad	1 V	None
Island L.	7/74	3	BT	Ad	1 V	None
Torso L.	7/74	1	Rb	Ad	1 V	None
	7/74	3	BT	Ad	1 V	None
Todd L.	7/74	34	BT	Ad	7 V	None
Sparks L.	7/74	60	BT	Ad	12 V	IPN
Elk L.	9/73	30	BT	Ad	6 V	IPN
	6/74	60	BT	Ad	12 V	IPN
	6/75	25	BT	Ad	5 V	IPN
	6/75	25	K	Ad	5 V	None

Table 8 (continued)

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Hosmer L.	6/74	16	AS	Ad	5 V	None
	9/74	5	BT	Ad	1 V	None
	10/74	2	BT	Ad	1 V	None
Big Lava L.	6/73	7	BT	Ad	2 V	None
	9/73	60	BT	Ad	12 V	IPN
	10/77	9	BT	Ad	2 V	None
Little Cultus L.	6/74	60	BT	Ad	12 V	None
	9/74	65	BT	Ad	13 V	None
	6/75	50	BT	Ad	10 V	None
	10/76	60	BT	Ad	12 V	None
N. Twin L.	10/74	10	K	Ad	2 V	None
	10/76	60	K	Ad	12 OF	None
	10/77	60	K	Ad	12 V	None
S. Twin L.	10/77	50	K	Ad	10V 4 S 12 OF	None
Charlton L.	8/74	45	BT	Ad	9 V	None

Table 8 (continued)

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Crane Prairie Res.	6/74	45	Rb	Ad	9 V	None
	6/74	5	K	Ad	1 V	None
	6/74	3	Co	Ad	1 V	None
	6/74	8	BT	Ad	2 V	None
	6/75	60	BT	Ad	12 V	None
Irish L.	8/74	8	BT	Ad	2 V	None
Taylor L.	8/74	15	BT	Ad	3 V	None
Waldo L.	12/76	26	BT & K	Ad	5 V	None
East L.	9/73	5	Br	Ad	1 V	None
	6/73	20	Rb	Ad	4 V	None
	6/73	15	BT	Ad	3 V	None
	7/73	70	BT	Ad	14 V	None
	10/73	120	BT	Ad	24 V	None
	10/73	210	BT	Ad	42 OF	None
	10/73	60	BT	Ad	12 V	None
	10/73	209	BT	Ad	40 OF	None
	11/73	156	BT	Ad	30 OF	None
	11/73	49	Br	Ad	10 OF	None

Table 8 (continued)

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Paulina L.	12/76	60	BT	Ad	12 OF	None
	10/78	60	K	Ad	12 V 5 OF	None
Wickiup Res.	6/74	4	Rb	Ad	1 V	None
	6/74	13	Br	Ad	3 V	None
	6/74	4	Co	Ad	1 V	None
	6/75	30	K	Ad	6 V	None
	6/75	30	Rb & Br	Ad	6 V	None
	9/77	12	K	Ad	2V 2 S 1 OF	None
Gold L.	8/74	64	BT	Ad	13 V	None
	8/74	10	Rb	Ad	2 V	None
	11/76	60	BT	Ad	12 V	None
	11/76	158	BT & Rb	Ad	31 V	None
	5/77	NR[6]	BT & Rb	Ad	V S OF	None
Odell L.	12/75	20	K	Ad	4 OF	None
	10/76	60	K	Ad	12 OF	None

Table 8 (continued)

Lake, River or Reservoir	Date	No. Fish Sampled	Species	Stage of Development	No. of Pools of Tissues or Fluids Sampled	Virus Isolated
Diamond L.	10/71	12	Rb	Ad	3 V	None
	5/74	90	Rb	Ad	12 V 5 OF	None
	5/75	50	Rb	Ad	10 OF	None
	4/78	60	Rb	Ad Br	12 V or OF	None
Fish L.	9/73	60	BT	Ad	12 V	IPN
Four Mile L.	10/73	60	BT	Ad	12 V	None
Mann L.	9/74	45	BT	Ad	9 V	None
	9/74	15	Rb	Ad	3 V	None
	5/77	60	Ct-Lah	Ad	12 OF	None
	5/78	25	Ct-Lah	Ad Br	5 V	None
	5/78	60	Ct-Lah	Ad	5 V 12 OF	None
Three Mile Res.	5/77	35	Rb-Red B	Ad	9 V 9 OF	None
	5/78	4	Rb-Red B	Ad	1 OF	None
Sid Luce Res.	8/76	15	Rb	Ad	3 V	None
Friday Res.	8/76	45	Ct	Ad	9 V	None

Table 8 (continued)

1. A tributary of the Umpqua River.
2. A tributary of the Coos River.
3. A tributary of the Coquille River.
4. A tributary of the Elk River.
5. Fish were held at Klamath Hatchery.
6. NR - not recorded.